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Stability Indicating RP-HPLC Method Development and Validation for the Analysis of Tizanidine in Bulk and Pharmaceutical Formulation

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Abstract

Background: A stability analysis is a critical aspect for figuring out the quality and also the purity of drug products under many stress conditions.

Introduction: The method aimed to develop a stability-indicating RP HPLC method just for the quantitative evaluation of Tizanidine (TZN) and to perform research of TZN below a variety of stress conditions and to validate the developed method according to the guidelines given by ICH Q2 (R1).

Method: Most of the assessment details were performed using Phenomenex kinetics C_{18} column (250 mm 4.6 mm id, 5 µm particle size) with the solvent method of water: methanol within the ratio of (50: 50) and also by adjusting the pH to 4 using orthophosphoric acid, and a detection wavelength of 318 nm. Forced degradation study was done by owing TZN APIs to degradations for example acid, alkaline, oxidative, thermal, and photolytic conditions to gauge the interference of degradants.

Results: The retention period of TZN was observed at 3.863 min. The suggested method was observed to become linear across the concentration assortment of 2-10 µg mL^{-1} for TZN. The regression coefficient (r^2) was found 0.9996 respectively, which is perfect for the acceptable bounds. The LOD was 0.30 µg mL^{-1}, likewise, LOQ was found to be 0.91 µg mL^{-1} respectively.

Conclusion: The method was discovered to become uncomplicated, exact, and accurate plus its ideal for any routine analysis and experiments of dosage forms containing TZN.

Keywords – Tizanidine, RP-HPLC, Stability studies, Forced degradation studies.

1.0 INTRODUCTION

Pharmaceutical product stability is a complex process collection that requires considerable time, expense, use, and scientific expertise to develop pharmaceutical formulation effectiveness, quality, and safety [1]. Any alteration that occurs after its preparation in a pharmaceutical product that adversely affects a patient's fitness for use in the quality of the product is of interest in the stability screening of pharmaceutical researchers and regulators [2]. The pharmaceutical stability test is defined according to the ICH guidelines is a standard test conducted in pharmaceutical products to demonstrate that drug quality is subject to various environmental factors such as humidity, temperature, and light for setting the drug test period or a pharmaceuticals shelf life and to recommend a good storage condition [3]. The USP defines the stability of the pharmaceutical product as "extension within certain limits" and uses the same characteristics and attributes as it had when its products were made [4]. The stability of active drugs and formulation is determined at an early phase of the drug development process [5]. The identity, strength, purity of components, as well as the stability and pharmaceutical analysis of manufactured products are essential in determining and ensuring [6]. Chemical product stability plays a vital role in substance degradation reactions such as racemization, hydrolysis, decrease, and oxidation. The steadiness of the pharmaceutical product too considerably affects factors including reaction mixture concentration, catalysts, radiation, pH, raw materials, and the period between product development and use. Physical changes, including vibration, temperature changes such as freeze, shear, and that, can impact drug stability, leading to the loss of the active pharmaceutical component [7]. Forced degradation, such as deterioration of drugs and drug components under conditions that are relatively higher than accelerated conditions, assess the stability of the molecules and therefore produce degradation products [8]. A pharmaceutical product stability test is designed to ensure the effectiveness, safety, and quality of the active drug substances and dose type and to assess shelf life or date of expiration to support branded claims [9]. Studies on stability must be carefully performed in compliance with legislation regulated by ICH, WHO, and similar bodies [10].

1.1 Importance of stability testing [11-13]

1. Determining conditions of shelf life and processing for the development of new products.
2. Toxic products may be formed during the decomposition of active drugs.
3. Ensuring that the brand is fit for use as long as they are in the market with all functionally acceptable attributes to protect the manufacturer's reputation.
4. To ensure that no modifications in the production have been implemented that can negatively impact product stability.
5. It offers a database that can be of value for current product development when choosing excipients, formulations, and closure schemes for containers.
6. Developing an understanding of API's degradation

that can affect the quality of the pharmaceutical product.

7. It is the only way to assures whether or not the drug meets the acceptance criteria.

1.2 Factors affecting drug stability

1. **Temperature**
   The stability of a drug substance is affected by changes in temperature when temperature increased it causes an increase in the hydrolysis rate of drugs [14].
2. **Moisture**
   Some physical and chemical dosage changes, when the water-soluble solid dose is absorbed into any moisture surface and therefore loses its properties [15].
3. **pH**
   The deterioration rate of hydrolyzed solution drugs is influenced by pH, to reduce the effect, drugs are formulated using buffers at the pH of optimum stability [16].
4. **Excipients**
   Starch and povidone excipients have greater water content and affect stability by enhancing the formulations of water content. Also, there are chemical interactions between excipients and drugs that lead to a reduction in instability [17].
5. **Oxygen**
   Oxygen presence facilitates oxidation in some products. Products with a higher decomposition rate are stabilized when exposed to oxygen by substituting carbon dioxide and nitrogen for oxygen in the storage container [18].
6. **Light**
   When exposed to light, the rate of decomposition increases. Certain drugs are photosensitive and their stability can be measured when exposed to light or stored in the dark by comparing their stability. Photosensitive medicines must be packed in a glass amber bottle and held in a dark place [18].

1.3 TZN [19-21]

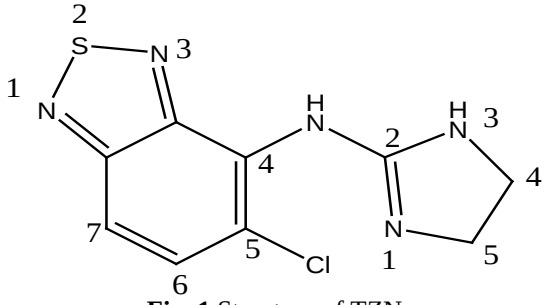

Fig. 1 Structure of TZN

TZN is a mostly old muscle tissue relaxant that has long been properly used at the therapy of serious liver injuries, several of that are living-threatening. TZN is a fast-acting drug used-to alleviate muscle tissue spasms when had to carry out a variety of pursuits. It performs when the alpha 2 adrenergic receptor agonist and also relieves muscle tissue spasticity, allowing regular daily things to continue. Within clinical trials, TZN hasn't been found to experience an immediate effect on neuromuscular junction or muscle tissue dietary fiber and possesses absolutely no sizable effect on monosynaptic spinal reflexes (comprised of touch somewhere between just one sensory neuron as well as one motor neuron). TZN has additionally been found to reduce the frequency of clonus as well as spasms.

2.0 EXPERIMENTAL

2.1 Chemicals and Reagents

Pure drug TZN was received from Sun Pharmaceutical Industries Ltd, Baroda, Gujarat as a gift sample. The marketed formulation (Tizan-2) containing 2 mg of TZN ended up being acquired from the Pharmacy outlet. HPLC methanol and water grade were purchased from Merck Ltd., Mumbai, India. Orthophosphoric acid from Merck Ltd., Mumbai, India. Concentrated hydrochloric acid (HCl) from Yarrow Chem Products, Mumbai. Sodium hydroxide (NaOH) from Yarrow Chem Products, Mumbai, and Hydrogen peroxide (H_2O_2) from Yarrow Chem Products, Mumbai.

2.2 Instrumentation

All the analytical studies were performed on the HPLC instrument (Shimadzu, Japan) equipped with an SPD 20A UV-visible detector and LC-20AT pump, manual Rheodyne injector with 20 µl loop, Phenomenex Kinetics C_{18} column (250 mm × 4.6 mm, 5 µm) and LC solution software.

2.3 Mobile phase Selection:

Several rigorous assessments have been carried out to improve the mobile phase. Various solvents for example methanol, water, and acetonitrile in various proportions and also several pH quantities of mobile phase ratios as well as buffer solutions to generate a sharp peak and baseline separating of the analyte with no intervention from the excipient. Good peak shape and tailing-free were achieved with the mobile phase, Water: Methanol (50: 50 v/v) of pH 4 using orthophosphoric acid in isocratic condition.

2.4 Preparation of solutions

2.4.1 Preparation of mobile phase

50 mL of methanol and 50 mL water (total 100 mL) had been taken and mixed, the pH of the solution was altered to four by adding few drops of orthophosphoric acid then filtered using Whatman's filter paper before sonication.

2.4.2 Preparation of TZN standard stock solution (10 µg mL^{-1})

10 mg of TZN was accurately weighed and then kept in a completely clean and dry 10 mL volumetric flask. It is then dissolved in enough amount of mobile phase and the volume was made up to the mark to attain 1000 µg mL^{-1} concentration (I stock). 1.0 mL of the above-mentioned solution was transferred into a completely clean as well as a dry volumetric flask of 10 mL and also the amount was loaded up with the mobile phase to the mark to obtain a concentration of 100 µg mL^{-1} (II stock). 1.0 mL of the solution taken from the II stock was then transferred into other 10 mL volumetric flask as well as the amount was

filled up to the mark with mobile phase to obtain 10 μg mL^{-1} a concentration.

2.4.3 Preparation of sample stock solution

20 TZN tablets have been taken then powdered. And approximately 10 mg of TZN was weighed effectively and kept directly into a thoroughly clean 10 mL volumetric flask after which the contents were dissolved with an adequate quantity of mobile phase. The solution was then sonicated for fifteen min and also the last volume was made up to 10 mL. This solution was then filtered with the aid of the Whatman filter paper. An aliquot of the supernatant solution was suitably diluted to obtain a concentration of 10 μg mL^{-1} of TZN.

3.0 METHOD VALIDATION

After the development of the method, validation of the developed method for the estimation of TZN was performed as per the ICH guidelines [22, 23]. The evolved HPLC method needs to be validated by different parameters to make sure that the overall performance attribute on the strategy fulfills the demands just for the planned purpose [24].

The following parameters had been carried out with the method validation [25, 26]:

3.0.1 System suitability

Method suitability is examined to evaluate the evolved chromatographic circumstances that are designed for analysis. A portion of 10 μg mL^{-1} of TZN standard solution was added to a clean and dry volumetric flask of 10 mL and it was dissolved with the help mobile phase up to the mark to acquire a concentration of 10 μg mL^{-1}.

3.0.2 Specificity

Specificity will be the method ability to stay unaffected within the presence of some other part which might be likely to be existing, these comprise impurities, degraded diluents, and products. Specificity was completed to make sure that the parts which are existing don't impact the sample being analyzed. 20 μl of standard solution and diluents had been introduced directly to the HPLC system and also the peaks are determined.

3.0.3 Linearity

The method linearity is the ability of its to offer test results that are specifically proportional to the analyte concentration within the sample. Linearity analysis was carried out from 2- 10 μg mL^{-1} for TZN.

3.0.4 Accuracy

Accuracy of the method or process is determined when the closeness of the test result received by that method match up against the true value. Accuracy was attained by the release of known concentrations of standard drugs. The method's accuracy was assessed by measuring the data of recovery of the identified quantity of analytes added.

3.0.5 Precision

The precision of the analytical method is the level of agreement between the specific examination results when the process is uniformly applied to several samples aided by the homogeneous test, precision of the analysis methods commonly expresses as relative standard deviation.

3.0.6 Limit of detection (LOD)

The limit of detection of the method is the minimum quantity of drug components that can easily be evaluated, moreover not generally quantified being a precise worth underneath the defined experimental factors.

3.0.7 Limit of quantification (LOQ)

The quantification limit of the method will be the least level of analyte which may be quantified precisely and accurately.

3.0.8 Robustness

The robustness of the analytical technique is a degree of the ability of its to stay unaltered by some small variants for instance flow rate modifications, some alteration in the ratio of the mobile phase, and changes in the wavelength.

4.0 FORCED DEGRADATION STUDIES STABILITY-INDICATING RP-HPLC METHOD FOR TZN.

4.0.1 Mobile phase preparation

50 mL each of methanol and water (total 100 mL) had been mixed and utilizing orthophosphoric acid and the solution pH was adjusted to 4. The solution was after that filtered by using Whatman's filter paper and this was kept in the sonicator for 15 mins ahead of work with to get rid of bubbles.

4.0.2 TZN standard stock solution preparation (100 μg mL^{-1})

10 mg of TZN was correctly weighed as well transferred straight into a volumetric flask of 10 mL. The powdered was then liquefy with a sufficient level of mobile phase and then finally diluted up to the mark with the same solvent. The solution was filtered. An aliquot of the supernatant solution ended up being diluted to obtain a concentration of TZN 100 μg mL^{-1}.

4.0.3 Acid hydrolysis

1.0 mL of an ordinary stock mixture of TZN was pipetted inside a thoroughly clean 10 mL volumetric flask. Then 1.0 mL of 0.1N HCl. The solution was preserved for 1 hour over a normal water warm bath as well as the heat range was taken care of at 60-70 ºC. The solutions were cooled at room heat which was counteracted with 0.1 N NaOH. Finally, the volume was adjusted approximately to the mark using the mobile phase solution to obtain a concentration of 10 μg mL^{-1}. Filtered the solution using a 0.45-micron filter paper. 20 μl was taken and after that introduced into the instrument and the chromatogram was determined.

4.0.4 Alkali hydrolysis

1.0 mL of standard stock solution of TZN was pipetted out there within a clean and also dried out 10 mL volumetric flask. Then 1.0 mL of 0.1N NaOH was added. The solution was kept for 1 hour in a water warm bath while keeping the heat at a temperature of 60-70 ºC. The solution was cooled to room temperature and it was counteracted with 0.1N HCl. Finally, the flask was filled with the mobile phase up to the mark to acquire the desired concentration of 10 μg mL^{-1}. Filter the solution using 0.45 micron. 20 μl was taken and was introduced into the HPLC instrument and the chromatogram was analyzed.

4.0.5 Thermal induced degradation

2.0 mL of standard stock solution of TZN was pipetted as well transferred inside a clean and dry 10 mL volumetric flask. Include 3 mL of diluents to it and also placed inside a hot air flow oven for 1 hour and maintained the temperature during 60-70 ºC. The solution was then cooled at room temperature and also volume was made up to the mark with the help of the diluent to get 10 µg mL^{-1} concentration. After that, the solution was filtered using a 0.45-micron filter paper. 20 µl of the solution was introduced into the HPLC system and the chromatogram was determined.

4.0.6 Oxidative degradation

1.0 mL of standard TZN from the stock solution was pipetted out to a volumetric flask 10 mL. Add 1.0 mL of hydrogen peroxide (H_2O_2) and also dilute for a 10 µg mL^{-1} concentration with the mobile phase. At an ambient temperature range, the solution had been kept apart for an hour. The solution was then filtered with 0.45 µ filter paper and 20 µl of the solution was introduced into the HPLC instrument and the chromatogram was analyzed.

4.0.7 Photodegradation

1.0 mL of standard stock solution of TZN was pipetted out within a clean and also dried out petri dish, it was subsequently placed inside a photo-stability chamber for five hours. The solution was cooled to room temperature. Finally, the volume was modified to 10 mL to get a 10 µg mL^{-1} concentration. Filtered the solution using 0.45 µ filter paper. 20 µl of the solution was introduced into the HPLC instrument and the chromatogram was determined.

5.0 Results And Discussion

5.1 Optimized chromatographic conditions

Peaks of TZN exhibits great resolution together with the solvent method of water: methanol within the ratio of (50: 50) and also by setting the pH to 4 utilizing orthophosphoric acid.

5.2 UV studies

The TZN sample was scanned at several wavelengths ranges from 200 - 400 nm range with the help of UV - spectrophotometer and the isosbestic point was determined at 318 nm, and the absorptivity values for the drug at optimized wavelength were calculated (fig. 2). The linearity data were given in table 1.

Table 1 UV linearity and wavelength studies

Concentration (µg mL^{-1})	Absorbance
10	0.5997
20	1.1066
30	1.6005
40	1.9987
50	2.5092

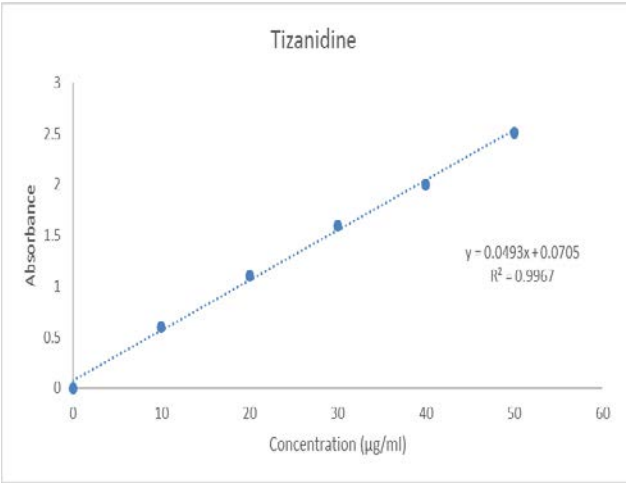

Fig. 2 Calibration graph for TZN

5.3 Stability Studies

a) Hydrolytic degradation in acidic conditions

The acidic condition applied to the active drug substances for 1 hour induced hydrolysis of TZN to about 10 %.

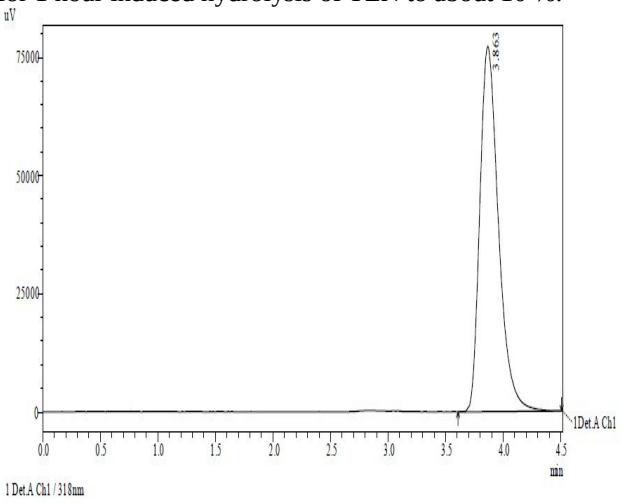

Fig. 3 Chromatogram of unstressed TZN

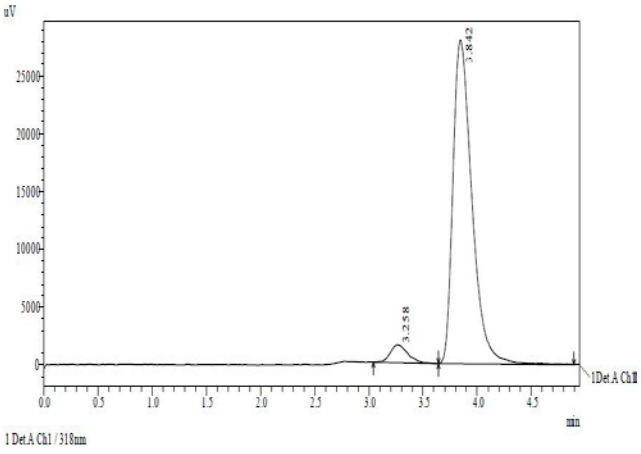

Fig. 4 Chromatogram of hydrolytic degradation of TZN in acidic condition

b) Hydrolytic degradation in alkaline conditions

The alkaline condition applied to the active drug substances for 1 hour induced hydrolysis of TZN to about 13%.

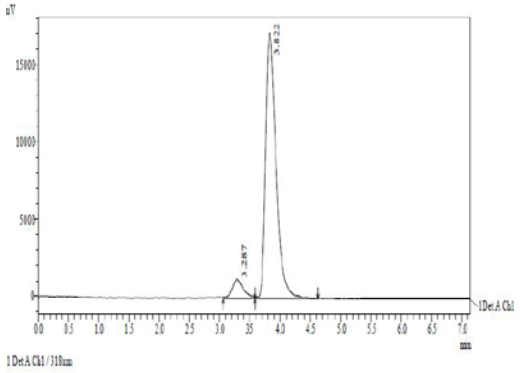

Fig. 5 Chromatogram for hydrolytic degradation of TZN in alkaline condition

c) Thermal induced degradation

The thermal condition applied to the active drug substances a hot air oven for 1 hour induced degradation of TZN to about 14%.

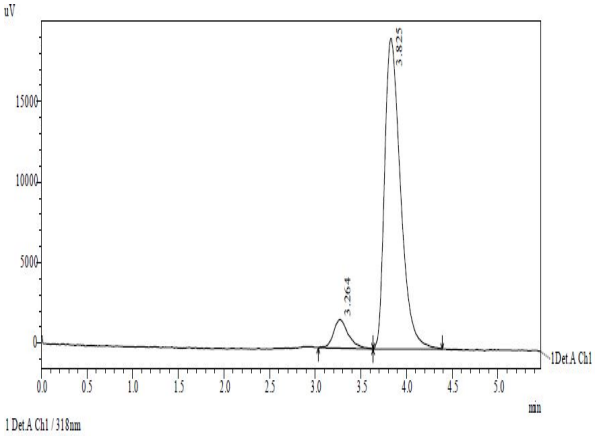

Fig. 6 Chromatogram for thermal-induced degradation of TZN

d) Oxidative degradation

The H_2O_2 applied on the active drug for 1 hour induced the oxidation of TZN to about 16 %.

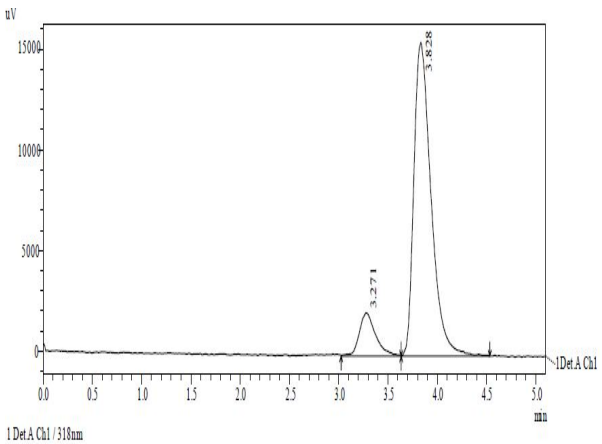

Fig. 7 Chromatogram for oxidative degradation of TZN

e) Photodegradation

Here the stress condition applied for 5 hours induced the degradation of TZN to about 2.5 %.

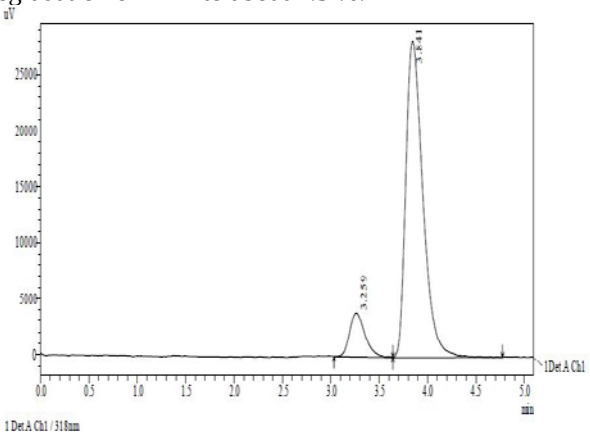

Fig. 8 Chromatogram for photodegradation of TZN

Table 2 Summarized data forced degradation studies

Parameters	Peak area of std. drug	Peak area degradant	% degradant
Acid degradation (0.1 N HCl)	453541	48456	10 %
Basic degradation (0.1 N NaOH)	335316	46419	13 %
Thermal degradation (Hot air oven)	348764	50095	14 %
Oxidative degradation	285191	45828	16 %
Photodegradation (Photostability chamber)	436644	11265	2.5 %

5.4 Method Validation

5.4.1 System suitability

Where the parameter like theoretical plates, symmetric factor, and resolution was recorded and found to be within the acceptance criteria.

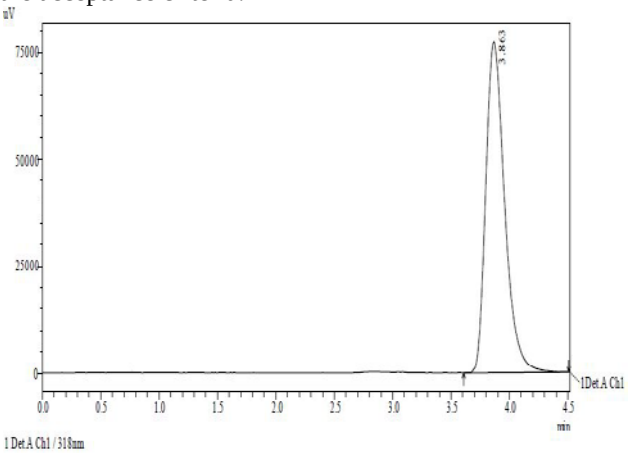

Fig. 9 Chromatogram for system suitability parameters of TZN

Table 3 System suitability test for TZN

Parameters	TZN
Retention time (min)	3.08 min
Tailing factor	0.953
Theoretical plates	2572

5.4.2 Specificity

There is no visible peak in chromatogram with diluent and excipients used in the formulation, which gives a good result for the specificity of the method.

5.4.3 Linearity

From the ranges of concentration (2 - 10 µg mL^{-1}) for TZN, it was observed that the regression coefficient (r^2) was found to be 0.99964 which falls within the limits. The result obtained was shown in the table. 4.

Table 4 Linearity data of TZN

Concentration (µg mL^{-1})	Peak area ± SD, n=3
2	210133 ± 148585.1
4	398026 ± 281444.1
6	607752 ± 429741.3
8	822907 ± 581877.5
10	1009755 ± 713997.5

5.4.4 Accuracy

The percentage recovery for TZN was found to be between 98 – 102 % which are well within the limits, indicating that there is no interaction from the drugs and even with the excipients found in the preparations.

5.4.5 Precision

Precision studies were performed by taking 3 different concentrations of TZN i.e. 2 µg mL^{-1}, 8 µg mL^{-1}, 10 µg mL^{-1}. The solution was introduced into the HPLC system and the chromatogram was analyzed. Precision was conducted both for intraday and interday and the % RSD was discovered to be < 2 %, indicating that the technique was precise.

5.4.6 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD values for TZN were determined and also was discovered at 0.30 µg mL^{-1}, similarly LOQ was found at 0.91 µg mL^{-1} respectively. This implies that the concentration with a reduced level is usually quantified with good precision and accuracy.

5.4.7 Robustness

In Robustness, it was discovered that generally there has been a small change in drug retention time and this falls within the acceptance criteria.

5.5 Application of RP-HPLC method for the assay of TZN in marketed formulation

Regarding the percentage purity**,** it was found to be within acceptance criteria 98 – 102 % which shows that the method can be employed for the routine analysis of TZN in marketed formulations. The degrading product generated as a consequence of stress did not interfere with the identification of TZN as well as subsequently may be considered as a stability-indicating. The method was discovered to be satisfactory, accurate, and reliable for stability scientific studies of TZN.

5.6 Discussion

A stability analysis is a critical aspect for figuring out the quality and also the purity of these medicines under many stress conditions. The objective was to develop a stability-indicating RP HPLC method just for the quantitative evaluation of TZN. To perform research of TZN below a variety of stress conditions and to validate the developed method according to the guidelines given by ICH Q2 (R1). The outcomes and findings from the studies are discussed as follows.

The TZN sample was scanned from a range of wavelength 200 - 400 nm using a UV - spectrophotometer and the isosbestic point was determined at 318 nm. For RP-HPLC many columns can be used for the analysis, but for our studies, the Phenomenex Kinetics C_{18} column (250 mm × 4.6 mm, 5 µm particle size) was preferred over other columns and the peak of TZN showed well resolution at a wavelength of 318 nm. For setting up stability studies strategy, as even the intermediate as well as the degradation products and solutions shouldn't interfere with any point during the analysis of the drugs. Also, after forced degradation has been attenuated to a 20-80 percent degradation, this may in some cases not be done even after longer-term exposure.

Forced degradation study was done by owing TZN APIs to degradations for example acid, alkaline, oxidative, thermal, and photolytic conditions to gauge the interference of degradants. The circumstances made use of for forced degradation study are discussed as follows.

Acid degradation, the acidic condition applied to the active drug substances for 1 hour induced hydrolysis of TZN to about 10 %. Hydrolytic degradation in the alkaline condition applied to the active drug substances for 1 hour induced hydrolysis of TZN to about 13%. Thermal induced degradation, the thermal condition applied to the active drug substances a hot air oven for 1 hour induced degradation of TZN to about 14%. Oxidative degradation, the H_2O_2 applied to the active drug for 1 hour induced the oxidation of TZN to about 16 %. Photodegradation, here the stress condition applied for 5 hours induced the degradation of TZN to about 2.5 %.

To produce precise, precise, specific, and suitable stability-indicating RP-HPLC method development as well as validation of TZN, various mobile phases had been utilized and also offered chromatographic state was observed ideal for the quantitative determination in presence of degradation products and impurities. The optimum mobile phase consisted of water: methanol (50: 50 v/v) of pH adjusted to 4 using orthophosphoric acid in isocratic condition. It was found to ideally resolve the peaks of TZN (t_R= 3.08 min) 0.5 mL min^{-1} effluent flow rate. The best analysis was found was with a UV detection wavelength of 318 nm, injection volume 20 µl, and ambient column temperature. The HPLC method was validated by using the following parameters according to the ICH guidelines. System suitability details for HPLC, where the parameter including theoretical plates, symmetric element, and resolution was recorded and also

determined to be inside the validation criteria. Specificity, there is no visible peak in chromatogram with diluent and excipients used in the formulation, which gives good specificity for the method. Linearity, observed over the concentration range of (2 - 10 μg mL^{-1}) for TZN was determined. The regression coefficient (r^2) was found to be 0.99964 respectively which was well within the acceptable limits. Accuracy, the percentage recovery for TZN was discovered to remain in between 98 - 102 % that is best within the limits, indicating essentially no interference belonging to the drug with each other or aided by the excipients contained in the formulation. Precision studies were performed by taking 3 different concentrations of TZN i.e. 2 μg mL^{-1}, 8 μg mL^{-1}, 10 μg mL^{-1}. It was injected into the HPLC instrument and the peaks were examined. Precision was performed both by intraday and interday and was found to be < 2 %, which demonstrates that the method was precise. Limit of detection The LOD values for TZN were calculated and found at 0.30 μg mL^{-1}, similarly LOQ was found to at 0.91 μg mL^{-1} respectively, which indicates that the concentration in small quantity can be driven with proper accuracy and also precision. The next validation parameters for the HPLC method are Robustness and it is seen that there were minor changes in the retention time of the drugs and also falls within the acceptance criteria.

Regarding the percentage purity**,** it was found to be within acceptance criteria 98 – 102 % which shows that the proposed method can be employed for the routine analysis of marketed formulations of TZN. The degradation product created due to pressure didn't interfere together with the detection of TZN as well as hence the method may be regarded as stability-indicating. Hence The technique was observed to be simple, precise, accurate, and is perfect for the regular evaluation of TZN. For the routine quality and stability determination of TZN this developed method can be easily and conveniently employed.

6.0 CONCLUSION

The stability studies for the assessment of TZN in bulk and pharmaceutical formulations were carried out successfully and the method was as well validated as per ICH guidelines. The developed method was validated for its suitability, specificity, accuracy, precision, linearity, and robustness.

Satisfactory recovery percentage and RSD values, therefore, indicated the applicability of the method developed for estimating TZN in pharmaceutical dosage forms. Maximum recovery is obtained by this developed method and the mean percentage recovery for each component was almost 100 %. The % RSD was shown < 2% for assay. The LOD of TZN was 0.30 μg mL^{-1}, similarly LOQ was 0.91 μg mL^{-1} respectively. The accuracy and also precision of the technique was found to be acceptable. There seemed to be absolutely no interference from the degradation products during the detection of TZN resulting from the stress studies.

The strategy was found to be simple, reliable, and consistent and is also ideal for the evaluation as well as stability studies of TZN dosage forms. The suggested procedure can be effectively and efficiently used for the stability studies of TZN.

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Conflict of Interest There is no conflict of interest among the authors.

REFERENCES

1. Arunachalam, A., Shankar, M., Stability studies. a review. *Asian J. Pharm. Anal. Med. Chem* 2008, 1(4), 184-195.
2. Naveed, S., Basheer, S., Qamar, F., Stability of a dosage form and forced degradation studies. *J. Bioequivalence Bioavailability* 2016, 8(4), 191-193.
3. Kommanaboyina, B., Rhodes, C.T., Trends in stability testing, with emphasis on stability during distribution and storage. *Drug Dev. Ind. Pharm* 1999, 25(7), 857-868.
4. Bhuyian, H.U., Rashid, H.A.R., Mohsin, M.d., Tahera, K.T., An overview. Stability study of pharmaceutical products and shelf-life prediction. *Eur. J. Biomed. Pharm. Sci.* 2015, 2(6), 30-40.
5. Sengupta, P., Chatterjee, B., Tekade, R.K., Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products. A comprehensive review. *Int. J. Pharm* 2018, 543(1-2), 328-344.
6. Bajaj, S., Singla, D., Sakhuja, N., Stability testing of pharmaceutical products. *J. Appl. Pharm. Sc.* 2012, 2(3), 129-138.
7. Maheshwari, R., Kar, M., Chourasiya, Y., Stability and degradation studies for drug and drug product. *Dosage Form Des. Consid* 2018, 7(6), 225-257.
8. Quadri, S.S., Sonwane, L.V., Poul, B., Kamshette, S., Review on stability-indicating assay methods (SIAMS). *PharmaTutor* 2014, 2(8), 16-31.
9. Komka, K., Kemeny, S., A modified error model for the assessment of the stability of pharmaceutical products. *Chemometr Intell Lab Syst* 2004, 72 (2), 161-65.
10. Blessy, M., Ruchi, D.P., Prajesh, N.P., Agrawal, Y.R., Development of forced degradation and stability indicating studies of drugs. A review. *J. Pharm. Anal* 2014, 4(3), 159-165.
11. Thorat, P., Warad, S., Solunke, R., Ashok, S., Anagha, B., Asha, S., Stability study of dosage form. *World J. Pharm. Pharm. Sci* 2014, 3(2), 1031-1050.
12. Suthar, N., Choudary, M., A review on stability studies of pharmaceutical products. *Int. J. Appl. Pharm. Biol. Res* 2017, 2(3), 67-75.
13. Agarwal, V., Sharma, D., Stability testing of active pharmaceutical and scientific innovation. *J. Pharm. Sci. Innovation* 2012, 1(2), 18-23.
14. Tollefson, A.E., Hermiston, T.W., Lichtenstein, D.L., Colle, C.F., Tripp, R.A., Forced degradation of Fast inhibits apoptosis in adenovirus-infected cells. *Nature* 1998, 392 (6677), 726-730.
15. Bakshi, M., Singh, S., Development of validated stability-indicating assay methods-critical review. *J. Pharm. Biomed. Anal* 2002, 28(6), 1011-1040.
16. Marin, A., Barbas, C., LC/MS for the degradation profiling of cough-cold products under forced conditions. *J. Pharm. Biomed. Anal* 2004, 35(5), 1035-1045.
17. Singh, S., Junwal, M., Modhe, G., Tiwari, H., Kurmi, M., Forced degradation studies to assess the stability of drugs and products. *Trends. Anal. Chem* 2013, 49, 71-88.
18. Singh, R., Rehman, Z.U., Current trends in forced degradation study for pharmaceutical product development. *J. Pharm. Educ. Res* 2012, 3(1), 54-63.
19. National Center for Biotechnology Information. PubChem. Tizanidine. Available from - https://pubchem.ncbi.nlm.nih.gov/compound/Tizanidine#section=Solubility [Accessed on 1 June 2020].
20. Semenchuk, M.R., Sherman, S., Effectiveness of tizanidine in neuropathic pain. An open-label study. *J. Pain* 2000, 1(4), 285-292.

21. British Pharmacopeia. Vol II. Medicines and Healthcare products Regulatory Agency, London 2016, pp 1064-1065.
22. Beckett, A.H., Stenlake, J.B., Practical Pharmaceutical Chemistry. 4thed. CBS publishers and distributors. New Delhi 1997, pp. 284-297.
23. Willard, H.M., Merritt, L.L., Dean, J.A., Settle, F.A., Instrumental method of Analysis.7th ed. CBS publication and distributors. New Delhi, 1986, pp. 513-514.
24. Gurudeep, C., Sham, K.A., Instrumental Methods of Analysis. 5th ed. Himalaya publishing home. Mumbai 2005, pp. 108-109.
25. Beckett, A.H., Stenlake, J.B., Practical Pharmaceutical Chemistry. 4th ed. CBS publishers and distributors. New Delhi. 1997, pp. 163-165.
26. International Conference of Harmonization ICH Q2 (R1). Validation of analytical procedures 2005: Text and methodology. Available from - https://www.ich.org/fileadmin/public%20website/ICH%20products/%20Guidelines%20Quality/%20Q2%20R1%20Guidline.pdf [Accessed 21 July 2020].