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# Development and Validation of an RP-HPLC Method for Estimation of Tizanidine Hydrochloride in Pharmaceutical Dosage Forms

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**ABSTRACT**

Tizanidine hydrochloride is a central muscle relaxant that acts as an agonist for centrally activating alpha-2 adrenoreceptors. It works for the treatment of spasticity, which is a neurological condition. Therefore, along with the increasing use of this drug, its quantification in the pharmaceutical formulation becomes essential for the drug quality control and regulatory purposes. A common way to detect it is using the tizanidine hydrochloride assay using liquid chromatography. In this study, qualitative and quantitative analysis-based RP-HPLC method was developed and validated for the estimation of tizanidine hydrochloride. The measurement of all tizanidine hydrochloride samples was performed on a C18 column, chromatographic separation was accomplished with an acetonitrile-phosphate buffer pH 7.5 (80:20 v/v) as the mobile phase at a flow rate of 1.5 mL/min during detection period. For detection purpose, use of UV detector was made at working wavelength of 230nm. The current procedure was verified in compliance with the ICH recommendations for a wide variety of parameters including specificity, linearity, precision, robustness and system suitability. The developed method shows suitable linearity at different concentration ranges with a correlation coefficient ($R^2$) of 0.998. The precision was confirmed by low relative standard deviations (%RSD) for intra- and inter-day analyses. The robustness of the approach was evaluated through slight intentional changes in the chromatographic conditions which indicated no significant influence on the results. In conclusion, the suggested method using RP-HPLC is appropriate for routine evaluation of pharmaceutical formulations and bulk tizanidine hydrochloride because of its simplicity and precision.

***Keywords:*** *Tizanidine hydrochloride, RP-HPLC method, precision, quantification etc.*

## 1. INTRODUCTION

Tizanidine hydrochloride (5-chloro-n-(4, 5-dihydro-1h-imidazol-2-yl)-2, 1, 3-benzothiadiazol-4-amine hydrochloride (John et al., 2011) is a skeletal muscle relaxant, acting within the central system, which was registered with the FDA in 1996 in the form of a 4 mg dosage and in 2000 a 2 mg supplement (Fig.1).(Kamen et al., 2008) As a pharmacological drug it is a derivative of imidazole and the chemical structure of this particular compound also features a phenyl substituent that is molecularly fused to a bicyclic imidazole moiety. Tizanidine works by selectively activating alpha-2 adrenergic receptors (Zhu et al., 2024a) that are primarily present in the spinal cord that is within the central nervous system. Once these receptors

are activated, they prevent the action of certain excitatory neurotransmitters such as glutamate which are known to mediate excitatory impulses that lead to muscular contractions. Thus, by minimizing the activity of these receptors, tizanidine decreases muscle tone, muscle spasms and maintains muscle strength. These drugs are known to show higher affinity for these receptors and work mainly at the spinal level hence there is improvement in muscle spasticity with reduced side effects on other body systems, thus it is used to treat many neurological disorders, multiple sclerosis, spinal cord injury, and because of its high efficiency and low side effects. It is available in both tablet and capsule forms, and dosing is typically initiated at lower levels to minimize side effects, gradually adjusting according to the patient's response.

In contrast to many other muscle relaxants, tizanidine is formulated to selectively act on the pathways located in the central nervous system (CNS), and therefore, its side effects such as sedation which are routinely observed with many muscle relaxants are less frequent with It .(Koyuncuŏglu et al., 1992)It is frequently administered to manage muscle tone, spasms, and stiffness, in order to enhance mobility and the overall life quality of patients suffering from these conditions. Though tizanidine is generally well tolerated, it can also be able to provoke some side effects, the most common ones being drowsiness, dry mouth, dizziness and weakness. (Malanga et al., 2008a)These effects are due to the central action of the drug. Less common, although more serious side effects include allergic reactions, hypotension and increase in liver enzymes (Semenchuk & Sherman, 2000)(Malanga et al., 2008b). It is important to assure its quality and efficiency, which is why appropriate analytical methods is required to comply with regulatory standard and quality standards. A number of methods have been reported for the analysis of tizanidine hydrochloride, there is a gap for a more stable, uncomplicated and validated RP-HPLC technique that meets current regulatory bodies guideline and standard. The study introduces such a method that is accurate, reproducible, and cost effective in terms of the amount of tizanidine applied as a sample.

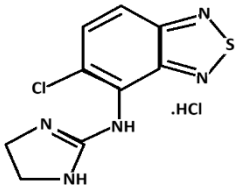



**Figure 1: Molecular structure of tizanidine hydrochloride**

## 2. MATERIAL AND METHOD

### Instruments:

Equipment includes a double beam UV-visible spectrophotometer with two 10 mm matched quartz cells and a SHIMADZU S-90 D digital balance. Globe Instrument Model 011G Digital PH Meter, Sonicator Model SVC 320, Waters Alliance All of the data from the Waters Alliance HPLC system was gathered and analyzed, and the HPLC system is designed for high-performance separation using Empower login software.

### Chemicals and Reagents:

This study involves tizanidine hydrochloride in Active Pharmaceutical Ingredient (API) form, which was used as the working standard for method development and validation. For the sample, Tizan®2 was procured from a nearby pharmacy store to determine whether the approach is applicable to commercial formulations.

The mobile phase and diluents were prepared using HPLC-grade water and HPLC-grade acetonitrile as the main solvents. The buffer solution was made using potassium dihydrogen phosphate ($KH_2PO_4$) as the buffering agent, and the pH was adjusted to 7.5 using potassium hydroxide (KOH). All reagents were of analytical grade, ensuring minimal interference in chromatographic analysis.

Filtration of the mobile phase and samples was performed using the whatman filter paper before analysis to remove any particulate matter and to ensure smooth column operation during chromatographic separation. The choice of high-purity chemicals and solvents, along with standardized procedures, contributed to the robustness and accuracy of the developed RP-HPLC method.

### Selection of Diluent:

The selection of the suitable diluent is a crucial step in method development to ensure accurate quantification of the drug. The solubility study for tizanidine hydrochloride was performed in various solvents, including water, methanol, ethanol, and acetonitrile (ACN), as well as in buffer solutions. The solubility was assessed visually to identify the solvent system that provided a clear solution without any precipitates.

In the all tested solvents, tizanidine hydrochloride exhibited moderate solubility in water, methanol, and ethanol, but it showed higher solubility in acetonitrile. Additionally, the combination of acetonitrile with phosphate buffer (pH 7.5) resulted in a clear and stable solution, which makes it the most suitable diluent for further analysis. This diluent not only ensured complete dissolution of the drug but also minimized the interference during chromatographic analysis. The prepared solutions were analyzed using UV-visible spectroscopy to confirm drug solubility and stability. The absorbance readings were recorded at the drug's maximum wavelength (λmax), and consistent sharp absorbance peaks were observed, which indicate that the drug is properly dissolved and the solution is uniform.

The chosen diluent (acetonitrile and phosphate buffer) was further evaluated for compatibility with the RP-HPLC system, which ensured that it did not interfere with the mobile phase and did not cause any damage to the column. The superior solubility and stability of tizanidine hydrochloride in this diluent contributed to the precision and accuracy of the developed RP-HPLC method.

**Standard stock solution preparation by using API:**

The solution was made by using an analytical balance, 1 mg of tizanidine hydrochloride (API) was precisely weighed before being put into a 10 mL volumetric flask. The medication was dissolved in a diluent, which was made by combining acetonitrile and phosphate buffer in a 40:60 (%v/v) ratio. After that, the mixture was sonicated for a few minutes, which ensured complete dissolution of the drug. To get a final concentration of 100 µg/ml, the solution was further diluted to the appropriate level using the same diluent, then this solution was filtered by using the whatman filter paper. The prepared stock solution was used for further dilutions and in the analysis during the method development and validation process.

**Preparation of Stock Solution of Marketed Sample**

To prepare the stock solution of the marketed sample (Tizan®2). Twenty tablets were weighed individually by using an analytical balance to determine their individual weights. The average weight of the tablets was calculated, which was found to be 90 mg. As per the label claim, each tablet contains 2 mg of tizanidine hydrochloride, which is considered to be its equivalent drug content. Based on the label claim and the average weight, A precise weight was taken of the quantity equal to 39 mg of tizanidine hydrochloride. Then it was dissolved in the chosen diluent which is acetonitrile and monopotassium phosphate buffer (PH 7.5) in the ratio of 60:40, and the solution was mixed thoroughly to ensure complete dissolution. Using the diluent, the solution's ultimate volume was brought down to 10 ml. which yielded a stock solution containing 100 ppm (100 µg/mL) of tizanidine hydrochloride. Further analytical and validation investigations were conducted using this generated stock solution.

**Method Validation**

The RP-HPLC method development and validation was performed as per ICH guidelines to ensure its reliability and suitability for routine analysis. The parameters were evaluated, which involve system suitability, precision (interday and intraday), Limit of the detection (LOD) and limit of quantitation (LOQ), linearity, and robustness.(Peris-Vicente et al., 2015)

**1. System Suitability**

System suitability tests are conducted to confirm that the HPLC system is suitable for the intended analysis. It ensures reproducibility and consistency of the chromatographic conditions.

This parameter ensures that the system operates within predefined specifications, which provide confidence in the obtained results. To perform the system suitability, the procedure involved injecting tizanidine hydrochloride standard solution into the HPLC the system, the method was considered suitable when retention time remains consistent and percent RSD was found to be ≤ 2. The results confirmed the system's suitability for analysis.(Kumar Bhardwaj, 2015)

**2. Precision (Intraday and Interday)**

Precision evaluates the closeness of agreement between multiple measurements under the same or varying conditions. It ensures the reproducibility and reliability of the method during repeated analysis.

For Intraday Precision, six replicates of a standard and sample solution were analyzed on the same day at different time intervals. and for Interday Precision, the same standard solutions were analyzed over consecutive days. The %RSD (relative standard deviation) for both intra- and interday precision was less than 2%, demonstrating excellent precision.(A. G. González et al., 2010)

**3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD is the lowest concentration of the analyte that can be detected but not necessarily quantified, whereas LOQ is the lowest concentration of the analyte that can be quantified with acceptable accuracy and precision(Chandran & Singh, 2007). Both parameters indicate the sensitivity of the method and were determined using the Standard Deviation of the Response and the Slope The detection limit (DL) may be expressed as: DL = 3.3* σ/S and for the limit of quantification LOD=10*σ/S, where σ is the response's standard deviation S is the calibration curve's slope, and the analyte's calibration curve can be used

to estimate slope S.

(Chandran & Singh, 2007) (international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ich harmonised tripartite guideline validation of analytical procedures: text and methodology q2(r1), n.d.)

**4. Linearity**

Within a certain range, linearity ensures that the method may produce findings that are exactly proportionate to the analyte's concentration. It ensures the method's reliability across a range of concentrations.

Linearity was performed by using standard solutions of tizanidine hydrochloride, which were prepared at five different concentrations within the range of ±20% of the standard solution, which is 100 µg/ml. After injecting these solutions into the system, a calibration curve was created by charting the peak regions versus the concentrations. The correlation coefficient (R2) was found to be 0.998, indicating excellent linearity.(Araujo, 2009; Suresh et al., 2010)

**5. Robustness**

Robustness ensures that method is reliable under the small, deliberate changes in analytical conditions. It evaluates how well the method can maintain consistent performance by minor variations, ensuring consistent performance.

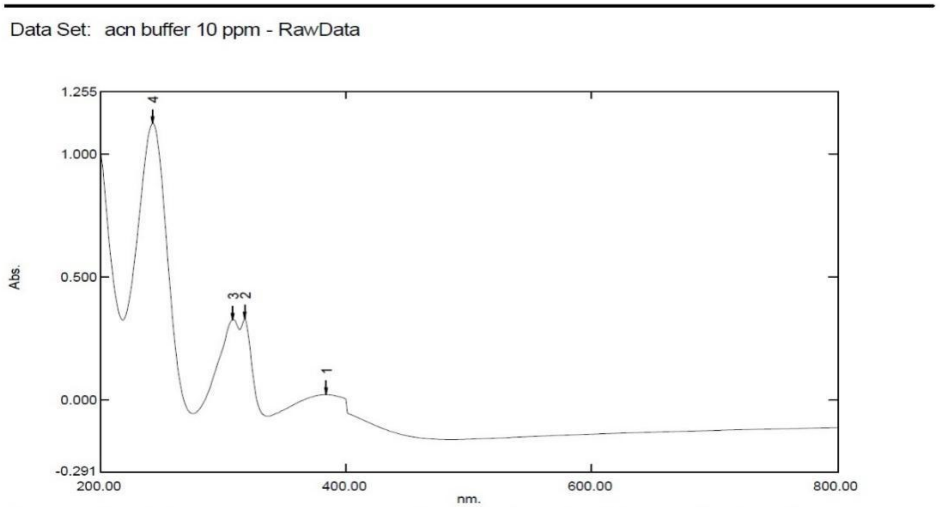
The robustness was checked by making minor changes to flow rate (±10%), mobile phase composition, and wavelength (±2nm). The system suitability parameters and peak characteristics were evaluated for each condition. and the result ensured that the method was robust, as no significant changes in retention time, resolution, or peak shape were observed (O. González et al., 2014)

## 3. RESULTS AND DISCUSSION

**Determination of Wavelength using UV-Vis Spectroscopy**

Finding the wavelength at which the analyte get absorbs the most strongly is the significance of λmax selection. ensuring high sensitivity It reduces the influence of other elements in the sample matrix.(Waghule et al., 2021)

The wavelength for the analysis of Tizanidine hydrochloride was determined using UV-Visible spectroscopy for which A solution of the drug was prepared at a concentration of 10 µg/mL using diluent made up of 80:20 (v/v) acetonitrile and phosphate buffer (pH 7.5). This prepared solution was checked at the wavelength in range of 200–400 nm by using a UV-Vis spectrophotometer to identify the wavelength at which maximum absorbance (λmax) was observed. The drug exhibited a maximum absorbance of 0.6 at a wavelength of 230 nm This was chosen as the wavelength of detection for the ensuing HPLC analysis. This 230 nm λmax was used consistently throughout the method development and validation processes, ensuring reliable and reproducible results (Fig. 2).




[Measurement Properties]
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Single

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

| No. | Wavelength | Absorbance | Description |
|---|---|---|---|
| 1 | 230.00 | 0.671 | |
| 2 | 215.00 | 0.354 | |
| 3 | 245.00 | 1.100 | |
| 4 | 300.00 | 0.214 | |
| 5 | 315.00 | 0.299 | |
| 6 | 330.00 | -0.044 | |
| 7 | 400.00 | -0.000 | |
| 8 | | | |

**Figure 2: Absorption spectra of standard 10ppm in ACN:Phosphate buffer(7.5) (80:20)**

**Calibration curve**

The curve for tizanidine hydrochloride was developed by plotting the concentration of the drug (in µg/mL) against the corresponding absorbance values, which were obtained using UV-Vis spectroscopy. Different dilutions The samples were obtained with concentrations ranging from 2 to 10 µg/ml using the diluent (acetonitrile: phosphate buffer, 80:20 v/v). The absorbance of each concentration was measured at 230 nm, the chosen wavelength (Table 1). A linear relationship was observed between concentration and absorbance within the specified range, and the calibration curve was generated.

**Table1: Absorbance of different concentration at 230nm**

| **Sr.no.** | Concentration(ppm) | Absorbance |
|---|---|---|
| 1 | 2 | 0.078 |
| 2 | 4 | 0.189 |
| 3 | 6 | 0.326 |
| 4 | 8 | 0.50 |
| 5 | 10 | 0.61 |

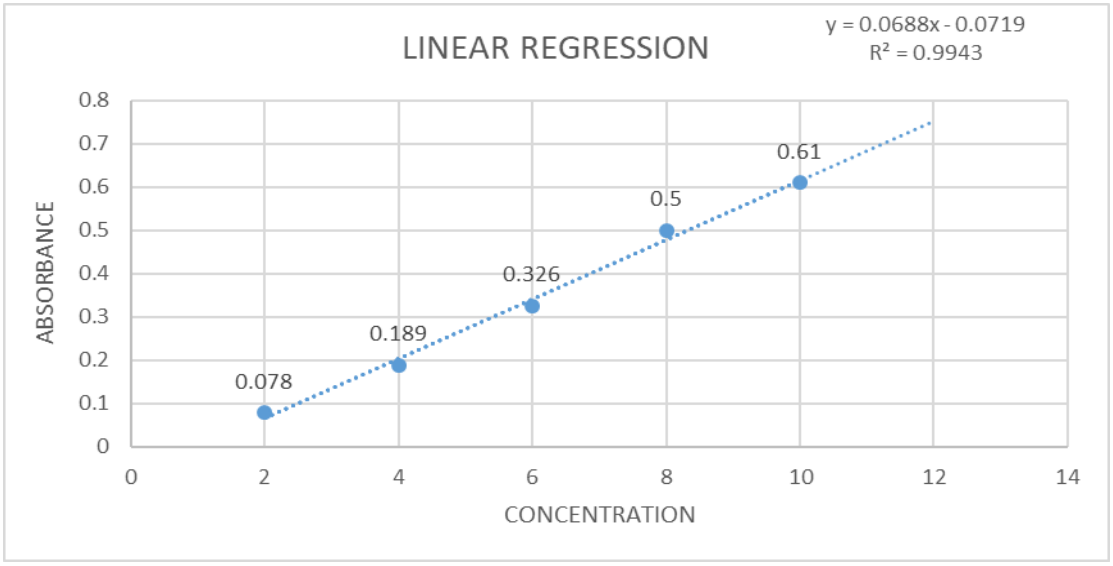



**Figure 3: Linear regression line obtained between absorbance vs concentration**

**The coefficient of correlation ($R^2$):**

Correlation coefficient has been found to be 0.9943 from the linear regression model suggest a high linear correlation between the drug's absorbance and concentration. The high value of $R^2$ proves that the method of UV-Vis is good for the quantitative assessment of tizanidine hydrochloride in proposed range (Fig.3).

**Method development:**

HPLC conditions:

- Mobile Phase: Acetonitrile: Phosphate Buffer (pH 7.5) in an 80:20 ratio
- Column: C18, 250 mm x 4.6 mm, 5 µm
- The rate of flow is 1.5 mL/min.
- The injection volume is 10 µL of the working solution at 100 ppm.
- **Detection Wavelength:** 230nm.
- **Column Pressure:** Approximately 1800 psi

**Result:** (as shown in fig 4) The retention time was found to be 2.631min, peak area 2601785, height of 188462 AU, USP Tailing 1.69 and USP Plate count of 2695.60.

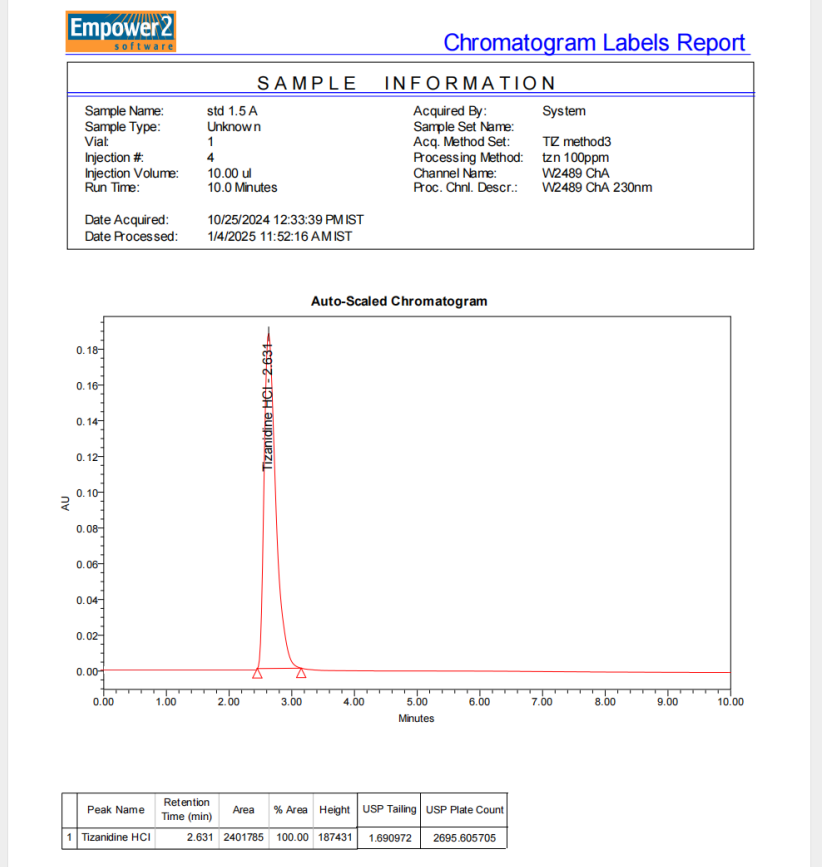



**Figure 4: peak obtained of std. tizanidine hydrochloride of 100ppm concentration**

**System suitability**

The standard solutions (100 PPM) have been administered in six repeat injections in system and calculated RSD was found to be less than 2%.

| Sr.no. | Retention time (min) | Peak area |
|---|---|---|
| **1** | 2.63 | 2692708 |
| **2** | 2.67 | 2584235 |
| **3** | 2.66 | 2714213 |
| **4** | 2.64 | 2653718 |
| **5** | 2.56 | 2638071 |
| **6** | 2.59 | 2604868 |
| **Mean** | 2.625 | 2647969 |
| **SD** | 0.042308 | 49881.03 |
| **RSD** | 1.611748 | 1.883747 |

**Table2: six replicate injection to test system suitability**

**Precision:**

The solution of standard 100 ppm of tizanidine hydrochloride was prepared. Two injections of this standard solution were administered to the HPLC system. (Tables 2,3 and 4) And five individual sample solutions of 100 ppm were prepared from

the marketed formulation and Each solution was injected twice in the system, resulting in a total of 12 injections (2 injections for each of the 6 preparations). The peak areas obtained for the Injections of both standard and sample were recorded. To assess precision, the percentage RSD was computed for both the standard injections and the sample injections.

**Table 3: precision of sample**

| SAMPLES | METHOD PRECISION | | INTERMEDIATE PRECISION | |
|---|---|---|---|---|
| | PEAK AREA | RESULT | PEAK AREA | RESULT |
| **spl01** | 2634244 | 100.69 | 2645462 | 101.7 |
| **spl02** | 2593770 | 99.14 | 2575741 | 99.02 |
| **spl03** | 2616719 | 100.02 | 2627714 | 101.01 |
| **spl04** | 2627984 | 100.45 | 2611258 | 100.38 |
| **spl05** | 2649673 | 101.28 | 2645888 | 101.71 |
| **spl06** | 2614455 | 99.93 | 2578496 | 99.12 |
| **Mean** | 2622807.5 | 100.2517 | 2614093.167 | 100.49 |
| **SD** | 19127.75611 | 0.732377 | 31389.57586 | 1.10 |
| **RSD** | 0.72928555 | 0.730539 | 1.200782599 | 1.09 |

**Table 4: RD in between MP and IP.**

| Sr.no. | INTRA DAY | INTERDAY |
|---|---|---|
| 1 | 100.69 | 101.7 |
| 2 | 99.15 | 99.02 |
| 3 | 100.02 | 101.01 |
| 4 | 100.45 | 100.38 |
| 5 | 101.28 | 101.71 |
| 6 | 99.94 | 99.12 |
| Mean | 100.26 | 100.70 |
| RD | 0.44 | |

**Limits of Quantitation (LOQ) and Detection (LOD):**

Using the formula, LOD and LOQ were calculated based on the response's standard deviation and slope. For LOD=3.3* σ/S and LOQ=10*σ/S. The procedure includes calculation of the standard deviation (σ) of the response which is the measure of response concentrate and peak area of the standard solution at the lowest concentration which was close to the detection limit. And the determination of the slope of the calibration (S) was obtained by preparing a calibration curve of peak area against the concentration which was used to obtain the linear regression to find out the slope (S) of curve (Table 5 and 6)

**Table 5a. Summary Output**

| **Regression Statistics** | |
|---|---|
| **Multiple R** | 0.991845616 |
| **R Square** | 0.983757726 |
| **Adjusted R Square** | 0.975636588 |
| **Observations** | 5 |
| **STANDARD ERROR OF INTERCEPT** | 2631.40470500073 |
| **SD OF INTERCEPT** | 5883.999797 |
| **SLOPE** | 30456.66 |

**Table 5b. Result**

| **Parameter** | **Concentration (ppm)** | **S/N** |
|---|---|---|
| **LOD= 3.3*residual SD/slope** | 0.63 | 3.51 |
| **LOQ=10*residual SD/slope** | 1.93 | 11.29 |

**Linearity:**

A series of standard solutions of tizanidine hydrochloride were prepared at concentrations ranging from ±20% of 100 ppm which covering 80 ppm to 120 ppm. These solutions were injected into the HPLC system under the optimized chromatographic conditions. And calibration curve was constructed by plotting the concentration of the standard solutions against their respective peak areas. ( Table 6 and Figure 5)

Over the measured concentration range, the linear regression equation and correlation coefficient (R2) were determined to be ≥ 0.998, suggesting high linearity.(Zhu et al., 2024b)

**Table 6: Linearity at different concentration**

| **SR.NO** | **Concentration (ppm)** | **Rt (min)** | **peak area** |
|---|---|---|---|
| 1. | 80 | 2.64 | 1908778 |
| 2. | 90 | 2.52 | 2235875 |
| 3. | 100 | 2.48 | 2593301 |
| 4. | 110 | 2.50 | 2903979 |
| 5. | 120 | 2.45 | 3097494 |

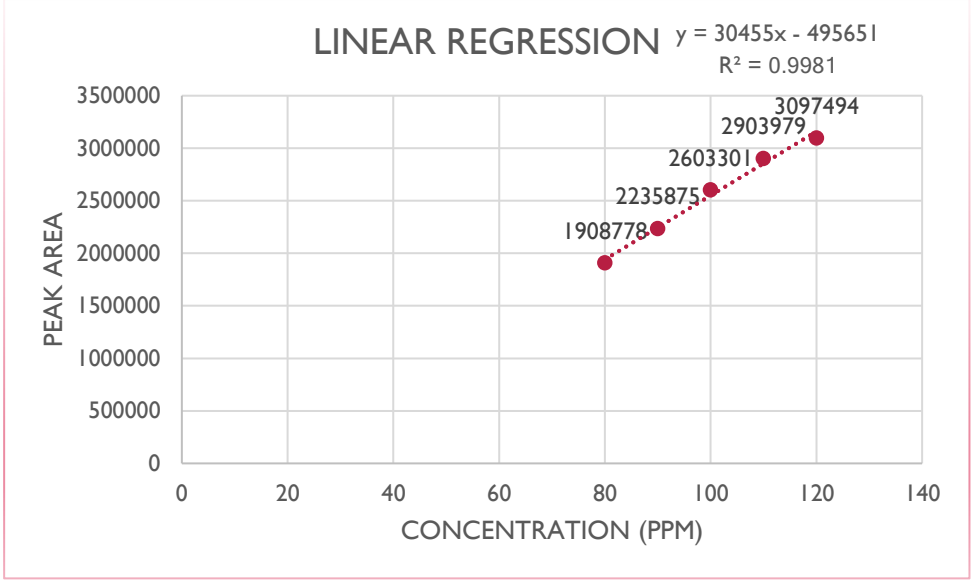



**Figure5: linear regression obtained between peak area and concentration**

**Robustness:**

The robustness was evaluated by making minors change to the analytical conditions and observing the influence on factors related to system appropriateness, including peak symmetry, theoretical plates, and retention time. Each modified condition was applied to analyze a standard solution of 100 ppm of Tizanidine hydrochloride (Table 7).

The following variations were tested:

1. Flow Rate: The flow rate was varied by ±10% (1.35ml/min and 1.65ml/min).
2. wavelenght: wavelenght was altered by ±2nm
3. Mobile Phase Composition: The ratio of acetonitrile to phosphate buffer (80:20 v/v) was altered by ±2% (78:22 and 82:18).

**Table 7: Results of Robustness**

| **Change in parameter** | **values** | **Rt (min)** | | **Peak area** | | **Assay%** |
|---|---|---|---|---|---|---|
| | | **STD** | **SPL** | **STD** | **SPL** | |
| **Flow rate (±10%)** | 1.35 ml/min | 2.97 | 2.93 | 2591145 | 2601044 | 101.18 |
| | 1.65 ml/min | 2.29 | 2.31 | 2621045 | 2573942 | 98.98 |
| **Wavelength (±2nm)** | 228nm | 2.57 | 2.55 | 2599289 | 2629670 | 101.96 |
| | 232nm | 2.50 | 2.47 | 2615349 | 2615971 | 100.82 |
| **Mobile phase composition (ACN: Buffer)** | 78:22 | 2.63 | 2.55 | 2611703 | 2599335 | 100.32 |
| | 82:18 | 2.5 | 2.47 | 2605349 | 2559471 | 99.022 |

## 4. CONCLUSION

The developed RP-HPLC technique for analysing tizanidine hydrochloride has been found to be clear, precise, and reliable, which makes it ideal for routine quality control of pharmaceutical formulations by using a C18 column acetonitrile and phosphate buffer (pH 7.5) in an 80:20 ratio were used as the method's mobile phase. The flow rate was 1.5 mL/min, and the detection wavelength was 230 nm.

System suitability parameters, including retention time, theoretical plates, and tailing factor, were within acceptable limits, confirming the reliability of the system. Precision studies demonstrated excellent repeatability, with %RSD values below 2% for both intraday and interday measurements. The method showed excellent linearity across the 80–120 ppm concentration range, with a correlation value of 0.998 and Sensitivity was confirmed through low LOD and LOQ values, making the method reliable even at low concentrations. The method's robustness was determined by introducing minor variations in flow rate, temperature of the column and the composition of the mobile phase. These changes had minimal impact on results, demonstrating the method's resilience and consistency. The technique was effectively used to analyze the marketed formulation (Tizan®2), confirming its accuracy and practicality for real-world applications.

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