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DEVELOPMENT AND VALIDATION FOR NEW ANALYTICAL METHOD OF TIZANIDINE BY RP-HPLC

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Abstract
A new HPLC method development and validation for the analysis of Tizanidine in bulk and pharmaceutical formulation was developed and validated. The method was validated according to ICH validation parameters like accuracy, linearity, precision, robustness. The UV spectra for the standard solution was scanned in the wavelength of 200nm to 400nm. The iso-bestic point was determined at 318nm. Peak of Tizanidine was resolved with the Mobile phase of 50:50 v/v of water and methanol and by adjusting the pH to 4.0 with ortho-phosphoric acid or sodium hydroxide and the satisfactory result in term of retention time, peak area, symmetry was obtained. All the analysis parameters were carried out using Shimadzu C18 column (15cm×4.6mm internal diameter, 5µ particle size) and the peak of Tizanidine showed well resolved at wavelength of 318 nm. The retention time of Tizanidine was found to be 5.574minute at the flow rate of 1.0 ml per minute and at 20µl of sample. The regression coefficient(r^2) was found to be 0.99972 for standard solution and 0.99964 for test solution, which is within the acceptance criteria. The percentage recovery of assay was 99.36% to 100.59%.
Index Terms: Tizanidine, RP-HPLC, Validation

1. Introduction:

Chromatography refers to the methods used for isolating, identifying, and analyzing the chemical constituents in complex mixtures. This technique is frequently employed, much like spectroscopy, and serves as a powerful tool for both preparatory and analytical applications, yielding compounds of very high purity. It involves the separation of mixture components based on their movement rates through a stationary phase (column) driven by a gaseous or liquid mobile phase; this is a straightforward definition of chromatography[1].

Chromatography is a technique utilized to separate a mixture by distributing its constituents between two distinct phases. The stationary phase remains fixed, while the mobile phase transports the mixture's components through the chosen medium. The stationary phase imposes constraints on various components, causing them to move more slowly than those in the mobile phase. The movement of these components within the mobile phase is influenced by the nature of their interactions with both the mobile and stationary phases. Variations in factors such as the solubility of specific components in the mobile phase and their affinity for the stationary phase result in differential movement speeds, thereby enabling the effective separation of the mixture's components.

Liquid chromatography was developed in 1906 by the Russian botanist Mikhail Tsvet during his efforts to isolate chlorophylls from plant extracts. This significant achievement earned him the title of the Father of Chromatography, more specifically the Father of Liquid Chromatography. Throughout the early 1900s, he continued to explore chromatography, primarily focusing on the separation of plant pigments such as carotenes, xanthophylls, and chlorophyll. The term 'chromatography,' meaning 'color writing,' was coined due to the distinct colors of these components (yellow, orange, and green). A novel form of chromatography emerged in the 1930s and 1940s, which has continued to be a vital and effective method for various separation and purification processes.[2]

A High Performance Liquid Chromatography (HPLC) method was created in 1960. It is the most widely used analytical method for identifying, quantifying, and separating any mixture of constituents. Non-volatile species such amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, medications, terpenoids, insecticides, antibiotics, steroids, organic metal species, and many inorganic chemicals can all be isolated with it. With the aid of relative affinity differences, the solvent in HPLC procedures is compelled to split samples into distinct constituents at pressure of up to 400 atmospheres. The HPLC column section has dimensions of 30 mm to 250 mm in length and 2.1 mm to 4.6 mm in width. Furthermore, the HPLC segments are made up of smaller, sorbent particles with a typical molecular size of 2.

2. Principle of HPLC

A separation column separates the stationary and mobile phases during purification. A granular substance containing microscopic porous particles serves as the stationary phase in a separation column. The mobile phase is the solvent or solvent mixture that is pushed through the separation column under high pressure. Through a valve with a linked sample loop—a tiny capillary or stainless steel tube—a syringe is used to feed the sample into the mobile flow regime from the pump to the separation column. The HPLC program generates a chromatogram upon completion of this operation or run. The chromatogram makes it possible to identify and measure the various chemicals.

Because of their interactions with the stationary phase, the components of a mixture subseque ntly migrate through the column at different speeds.

A appropriate detector recognizes a compound when it exits the column and is transmitted as a signal to the computer's HPLC software[3].

3. Tizanidine

Tizanidine is a frequently prescribed muscle relaxant that has been associated with infrequent cases of acute liver damage, some of which have resulted in fatalities[4].

Pharmacodynamics: Tizanidine is a fast-acting medication utilized to alleviate muscle spasticity when necessary for specific tasks. It functions as an agonist at Alpha-2 adrenergic receptor sites, mitigating symptoms of muscle spasticity and facilitating the performance of routine daily activities. In studies involving animal models, Tizanidine has not demonstrated direct effects on skeletal muscle fibers or the neuromuscular junction, nor has it exhibited a significant impact on monosynaptic spinal reflexes, which involve the interaction between a single sensory neuron and a single motor neuron. The occurrence of muscle spasms and clonus has been shown to be reduced by tizanidine. It exhibits a more pronounced effect on

polysynaptic reflexes, which entail the communication of multiple interneurons (relay neurons) with motor neurons that trigger muscle movement[5].

Adverse Drug Reactions: Drowsiness, dizziness, weakness, feeling nervous, blurred vision, flu-like symptoms, dry mouth, trouble speaking, abnormal liver function tests, runny nose, sore throat, urination problems, vomiting, constipation, or uncontrolled muscle movements are some of the adverse effects of TZN.

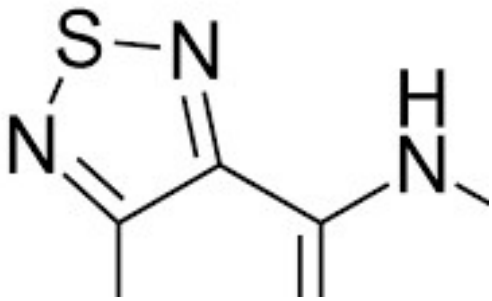


Figure 1: Tizanidine

4. Validation parameters recommended by FDA, USP and ICH are as follows

a. System Suitability

This is an essential component of liquid chromatography techniques employed to assess the suitability, efficiency, resolution, and reproducibility of the chromatographic method for analysis. The evaluation of the system's suitability involves the calculation of several factors, such as peak resolution, peak tailing, the number of theoretical plates, a nd overall efficiency[6].

b. Accuracy

The accuracy of an analytical method refers to how closely the obtained value matches the accepted true or reference value. Accuracy is expressed as a percentage recovery, which is determined by assessing the known added quantity of analyte (80%, 100%, 120%) in the sample, or by calculating the deviation between the average result and the accepted true value, factoring in the confidence interval. The International Conference on Harmonization (ICH) advises that accuracy should be evaluated by performing at least nine measurements across a minimum of three different concentrations within the specified range (i.e., three concentrations and three replicates for each concentration)[7].

c. Linearity

Linearity (within a specific range) is the capacity of an analytical technique to produce test findings that are precisely proportionate to the concentration of the sample analyte. According to the ICH Guideline, linearity should be evaluated using a minimum of five concentrations. An analytical technique's range is the difference between its upper and lower levels, and it is commonly measured with linearity, accuracy, and precision using the method procedure[8].

d. Precision

The level of consistency among measurements obtained from various samplings of the same uniform sample is referred to as the precision of the analytical method. Repeatability: The

repeatability of a testing procedure is determined by conducting complete independent assessments of the same uniform batch of material on designated samples, and this serves as an indicator of the method's precision under typical laboratory conditions. It represents a short-term analytical method conducted under identical operations[9].

e. LOD

The analytical limit refers to the minimum quantity of an analyte present in a sample that can be detected, but it is generally not reported as an exact measurement. Typically stated as the concentration of the analyte in the sample, the focus of the limit of detection (LOD) can be the signal-to-noise ratio (S/N) at 3:1[10].

f. LOQ

The quantification limit for a specific analytical method is defined as the lowest amount of a sample that can be accurately and precisely measured. The LOQ is typically derived from the determination of a signal-to-noise ratio (S/N ratio) of 10:1, by injecting standards that produce this ratio and exhibit an acceptable level of percent relative standard deviation[11].

g. Robustness

The robustness of an analytical procedure refers to its capacity to endure slight, deliberate var iations in method parameters such as pH, temperature, mobile phase composition and instrumental settings while demonstrating reliable performance under standard operating conditions. The robustness of an analytical procedure refers to its capacity to endure slight, deliberate variations in method such as pH, temperature, mobile phase composition, and instrument settings while maintaing reliability under standard operating conditions. The robustness of an analytical procedure refers to its capability to endure small, deliberate variations in method parameters (such as pH, temperature, mobile phase composition, and instrumental settings) while maintaining reliability under standard operating conditions[12].

5. Methodology

a. Mobile Phase: A total of 100 ml was prepared by combining 50 ml of methanol with 50ml of water. The pH of the mixture was adjusted to 4.0 by adding a few drops of orthophosphoric acid. Subsequently, the solution was filtered using Mobile phase filter paper and sonicated for 15 minutes before use.

b. Diluent: Mobile Phase

c. Preparation of Standard Solution: Weighed accurately 10 mg Tizanidine working standard into a 100 ml volumetric flask. Dissolve and diluted to volume with Mobile Phase. Dilute 1 ml of this solution to 10 ml with same solvent mixture and mix. (0.01mg/ml)

d. Preparation of sample solution: Accurately weighed 20 tablets were ground to obtain fine powder. Equivalent to 10 mg of Tizanidine sample were weighed and transferred to 100 ml of volumetric flask and dissolved in Mobile Phase. The flask volume was made up to mark with mobile phase and was shaken. Further 1 ml of the resulting solution was 10 ml with mobile phase. (0.01mg/ml)

e. Determination of Working Wavelength (λ max)

A volume of 20 µl of the TZN standard solution (0.01mg/ml) was introduced into the HPLC system at various wavelengths, resulting in a chromatogram with acceptable resolution at 318 nm.

f. Optimum Chromatographic Conditions

The peaks of TZN were distinctly separated using a solvent system composed of water and methanol in a 50:50 ratio, with the pH adjusted to 4.0 through the addition of orthophosphoric acid. The optimized chromatographic conditions are detailed below.

- **Column:** Shimadzu C_{18} Column (150mm ×4.6mm, internal diameter: 5µ particle size)
- **Mobile Phase:** Water and Methanol (50:50% v/v)
- **Flow Rate:** 1.0 ml/minute
- **Detector:** UV-Detector
- **Injection Volume:** 20µl
- **Wavelength:** 318nm
- **Oven Temperature:** 30ºC

6. Results and Conclusion:

The UV spectra of the standard solutions were analyzed within the wavelength range of 200 to 400 nm. The absorptivity values of the drug at the optimal wavelength were calculated, and the isobestic point was determined to be at 318 nm. The information regarding linearity is presented below.

S.N.	Concentration (µg/ml)	Absorbance
1.	10	0.123
2.	20	0.248
3.	30	0.373
4.	40	0.497
5.	50	0.624

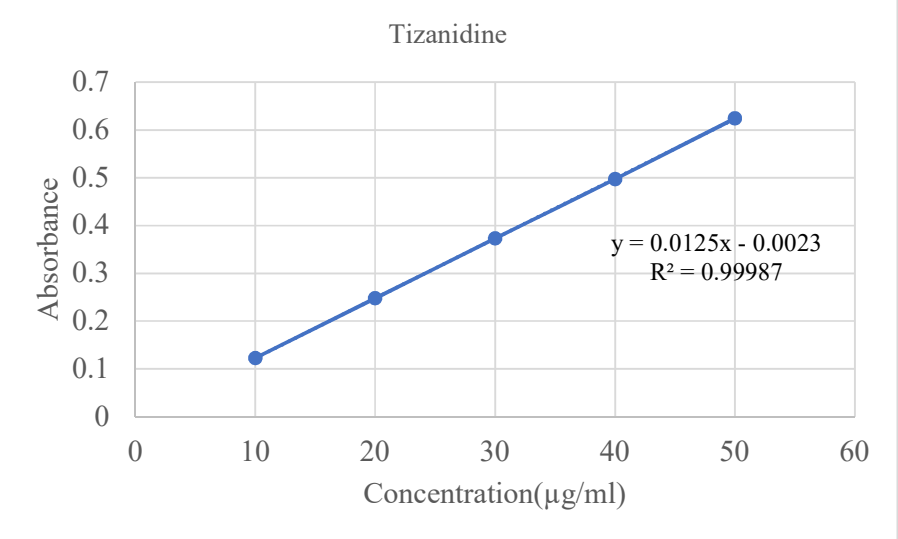


Fig.2: Calibration graph for Tizanidine

Based on the aforementioned experimental findings, it was found that a freshly developed technique to perform the simultaneous estimation of Tizanidine was straightforward, particular, and high resolution. Creating an HPLC method for Tizanidine quantitative qualification was the goal. The following is a discussion of the stud's findings and outcomes.

Using a UV Spectrophotometer, the Tizanidine standard sample was scanned at wavelengths between 200 nm to 400 nm, and the wavelength was fixed to 318 nm. When it comes to HPLC, columns come in an array of configurations. However, for our investigations, the Shimadzu C_{18} Column (150mm ×4.6mm, internal diameter: 5µ particle size) was chosen above other columns, and the peak of Tizanidine demonstrated good resolution at 318 nm.

Specificity: There was no visible peak on the bands when formulation's diluent and excipients were used, indicating a high degree of specificity for the recommended method.

Linearity: Linearity was observed over the range of 80% to 120% (80%, 90%, 100%, 110%, and 120%). It was discovered that the regression coefficient(R^2) was 0.99975 for Standard Solution and 0.99928 for Test Solution, which was substantially within the acceptable bounds.

Accuracy: Tizanidine Percentage recovery was determined to be between 99.36 to 100.59%, which is well within the allowed range and observed that there was essentially no drug interference with the formulation's excipients or medications.

Intermediate Precision: The Assay percentage of three analysts on different was found to be 99.58%, 98.84% and 99.19% respectively. The repeatability was 1.064 and the intermediate precision was 0.692, which are within the acceptance criteria.

Robustness: It is the another Validation parameter for the HPLC method, and it was observed that there was a slight variation in the drug's retention time that nevertheless met the acceptance criteria.

It was discovered that the technique is easy to use, accurate, precise, and appropriate for routine analysis of Tizanidine.

The approach that has been devised is simple and convenient to utilize for Tizanidine Tablet routine quality and stability determination.

An entirely novel HPLC technique has been developed and authorized for use in bulk as well as pharmaceutical formulation analysis of tizanidine. The procedure's robustness, accuracy, linearity, specificity and precision were all proven. For Tizanidine estimation in pharmaceutical formulations, the proposed method's suitability was verified by its low % RSD values and satisfactory percentage recoveries. This developed technique produces the highest recovery, with an average recovery of almost 100% for every component.

It was determined that the method's precision and accuracy were satisfactory. The technique was shown to be straightforward, accurate and exact; as such, it is appropriate for regular dosage form analysis and stability investigation with Tizanidine. The devised approach is simple to use and easy for routinely determining Tizanidine and stability.

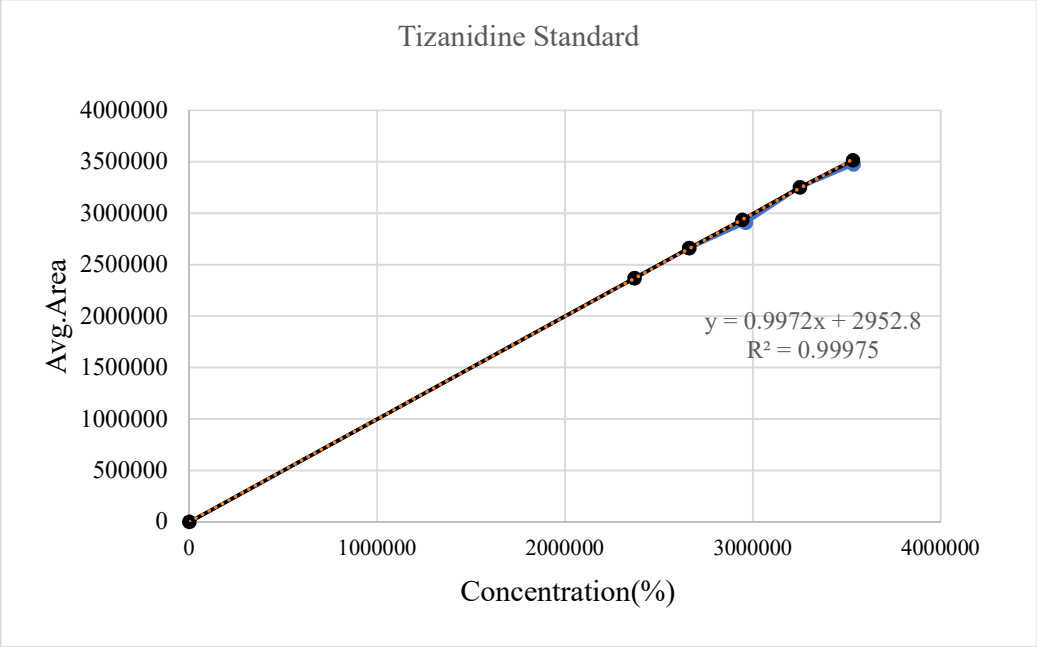


Fig. 3: Linearity curve of Standard

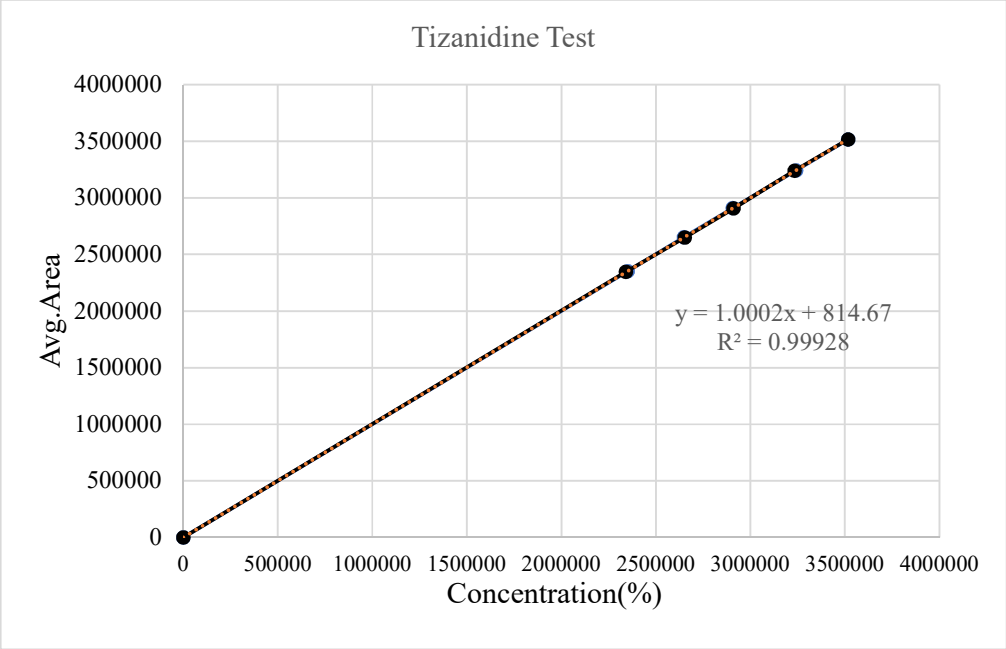
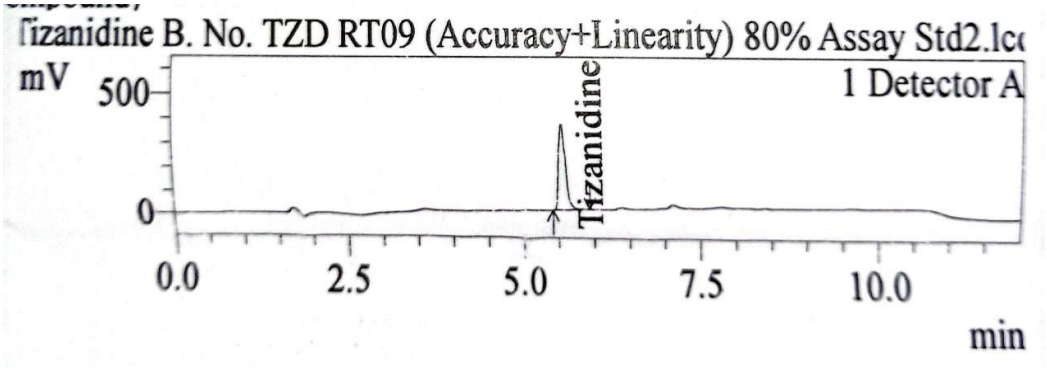


Fig. 4: Linearity curve of Test


Tizanidine B. No. TZD RT09 (Accuracy+Linearity) 80% Assay Std2.lc
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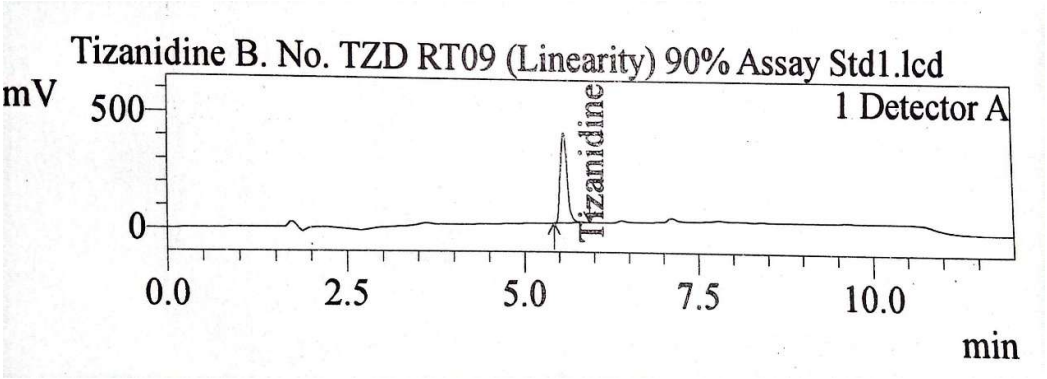
Tizanidine B. No. TZD RT09 (Linearity) 90% Assay Std1.lcd
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7.5
10.0
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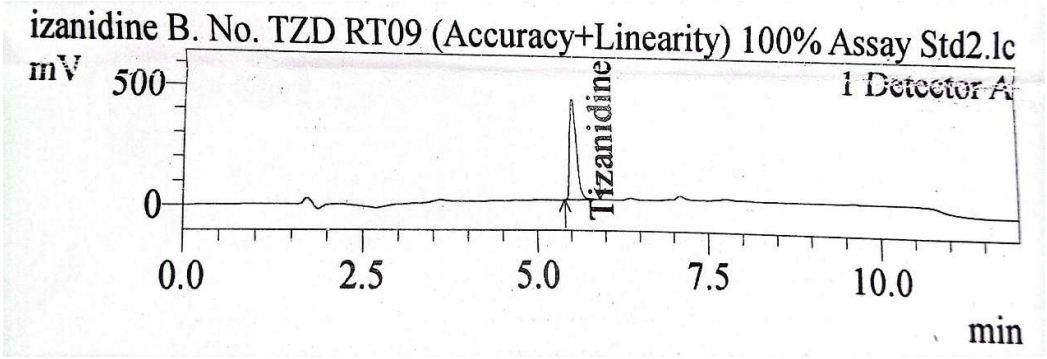
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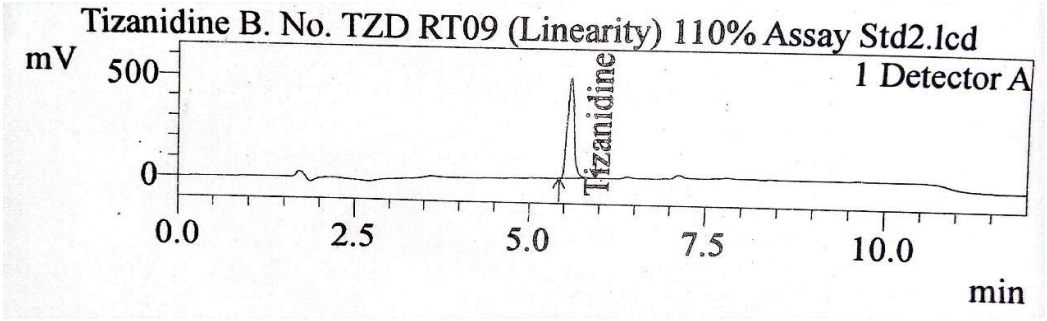
Tizanidine B. No. TZD RT09 (Linearity) 110% Assay Std2.lcd
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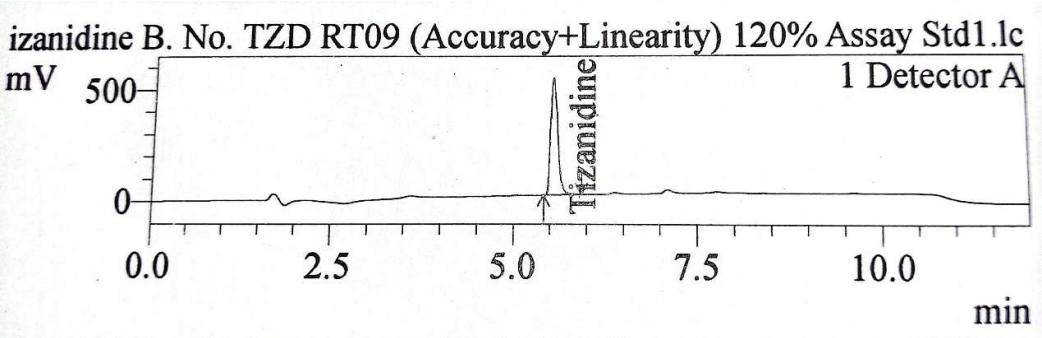

Fig.4 : Linearity of standard at 80%, 90%, 100%, 110% & 120%

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