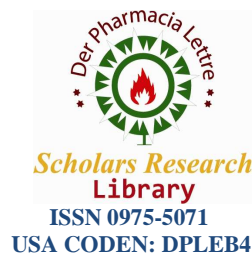




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Method development and validation of tradozone by RP-HPLC

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Tradozone, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Sunfire C18 (4.6 x 250mm, 5 μ m) column using a mixture of Methanol: Water (50:50% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 254nm. The retention time of the Tradozone was 2.165 \pm 0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Tradozone. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Tradozone, RP-HPLC, validation.

INTRODUCTION

Analytical chemistry-Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behavior of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter [1].

Chromatography-The chromatography was discovered by Russian Chemist and botanist Micheal Tswett (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin[2].

“Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system”.

Chromatographic Process-Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed

in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases.[4] High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods [3].

MATERIALS AND METHODS

METHODS IN CHROMATOGRAPHY- [5] According to nature of stationary and mobile phase: Solid- Liquid chromatography, Liquid-Liquid chromatography, Gas- Solid chromatography, Gas -Liquid chromatography. According to principle of separation: Adsorption chromatography: Gas Solid chromatography, Thin layer chromatography, Column chromatography, High performance liquid chromatography, Affinity phase chromatography, Hydrophobic Interaction chromatography (HIC) Partition chromatography: Gas liquid chromatography, Paper partition chromatography, Column partition chromatography, Based on modes of chromatography, Normal phase chromatography, Reversed phase chromatography Other types of chromatography, Size exclusion chromatography (SEC), Gel permeation chromatography, Gel chromatography, Gel Filtration, Gel permeation chromatography, Ion exchange chromatography, Chiral chromatography

High Performance Liquid Chromatography (HPLC)-The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography [6].

Types of HPLC techniques- Based on modes of separation, Normal phase chromatography, Reversed phase chromatography, Based on principal of separation, Adsorption chromatography, Partition chromatography, Ion exchange chromatography, Ion pair chromatography, Size exclusion or Gel permeation chromatography, Affinity chromatography, Chiral phase chromatography, Based on elution technique, Isocratic elution, Gradient elution, Based on scale of operation, Analytical HPLC, Preparative HPLC, Based on types of analysis, Qualitative analysis, Quantitative analysis[7].

Working Principle of HPLC -The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column [8].

Components of HPLC- Solvent, Solvent Delivery System (Pumps), Injector, Column, Detectors, Recorder (Data Collection). General HPLC column care. The correct use of an HPLC column is extremely important for the life time of a column and therefore for the benefit of your HPLC analysis. The following lines will give us some guidelines for the use, cleaning and storage of HPLC columns. These guidelines depend on the one hand on the nature of the chromatographic support (silica, polymers or others) and on the other hand on the surface chemistry of the corresponding stationary phase [9].

Detectors of HPLC-A- chromatography detector is a device used liquid chromatography (LC) to visualize components of the mixture being eluted off the chromatography column. There are two general types of detectors: destructive and non-destructive. The destructive detectors perform continuous transformation of the column effluent (burning, evaporation or mixing with reagents) with subsequent measurement of some physical property of the resulting material (plasma, aerosol or reaction mixture) [10-14].

The non-destructive detectors are directly measuring some property of the column effluent (for example UV absorption) and thus affords for the further analyte recovery. The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column.

ANALYTICAL METHOD VALIDATION

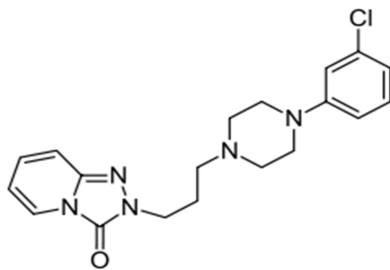
Method validation can be defined as per ICH "Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

ICH Method validation parameters-For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B). Specificity, Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantitation, Robustness, System suitability [18]-[19].

DRUG PROFILE-

[15]-[20] **TRADOZONE:**

Drug category: Ant depressive Agents, Second-Generation, Anti-Anxiety Agent, Serotonin Uptake Inhibitor,



Structure :Chemical name/ Nomenclature/IUPAC Name:2-{3-[4-(3-chlorophenyl)piperazin-1-yl]propyl}[1,2,4]triazolo[4,3-a]pyridin-3(2H)-one, Molecular Formula: C₁₉H₂₂ClN₅O, Molecular Weight :371.8641 g/mol, Official Pharmacopoeia :USP,BP,

Physicochemical Properties: Description (Physical State): white, odorless, crystalline powder, Solubility: freely soluble in water, Storage Conditions: Store at room temperature. Protect from temperatures above 104°F (40°C). Dispense in tight, light-resistant container (USP). Dosage: 300mg, Melting point: 87°C, pKa(strongest basic):7.09, Log P:2.9,

AIM AND OBJECTIVES

To develop new simple, sensitive, accurate and economical analytical method for the estimation of Tradozone and to validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Tradozone in dosage form.

EXPERIMENTAL WORK:

Preparation of standard solution: Accurately weigh and transfer 10 mg of Tradozone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3ml of the above Tradozone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol and Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water and Acetonitrile: water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water (50:50% v/v) respectively.

Optimization of Column: The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. Sun fire C18 (4.6 x 150mm, 5 μ m) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized Chromatographic Conditions: Instrument used: Waters HPLC with auto sampler and PDA Detector 996 model. Mobile phase ratio: Methanol: Water (50:50% v/v), Column: Sunfire C18 (4.6 \times 250mm)5 μ , Column temperature: 40°C, Wavelength: 282nm, Flow rate: 1ml/min, Injection volume: 10 μ l, Run time: 5min

VALIDATION:

Preparation of Mobile Phase: Accurately measured 500 ml (50%) of Methanol and 500ml of water (50%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

VALIDATION PARAMETERS:

System Suitability: Accurately weigh and transfer 10 mg of Tradozone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above Tradozone stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity Study of Drug:

Preparation of Standard Solution-Accurately weigh and transfer 10 mg of Tradozone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above Tradozone stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution: Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Tradozone sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3 ml of Tradozone above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Drug Solutions for Linearity: Accurately weigh and transfer 10 mg of Tradozone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10 ppm of Tradozone): Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (20 ppm of Tradozone): Take 0.2 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (30 ppm of Tradozone): Take 0.3 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (40 ppm of Tradozone): Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (50 ppm of Tradozone): Take 0.5 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure: Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient

RESULTS AND DISCUSSION

Table: Optimized Chromatogram (Standard)

S. no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Tradozone	2.165	576012	505117	1.41	5846

Observation: In this chromatogram, the peak is well separated and theoretical plate count is within the limit. So, this chromatogram is optimized.

Optimized chromatogram (sample):

Mobile phase ratio Methanol: Water(50:50% v/v), Column: Sunfire C18 (4.6×250mm) 5 μ , Column temperature: 40°C, Wavelength: 254nm, Flow rate: 1ml/min, Injection volume: 10 μ l, Run time: 5min.

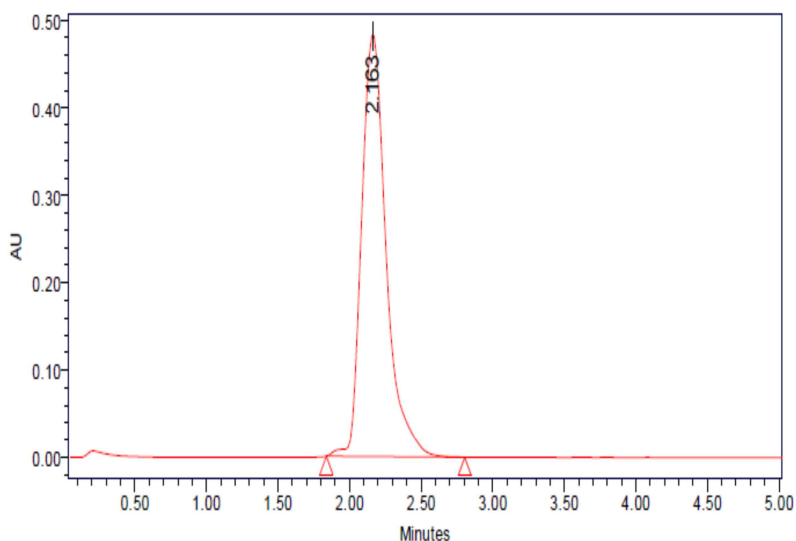


Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Tradozone	2.163	575224	483391	1.43	5839

Acceptance criteria: Resolution between two drugs must be not less than 2, Theoretical plates must be not less than 2000, Tailing factor must be not less than 0.9 and not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION:

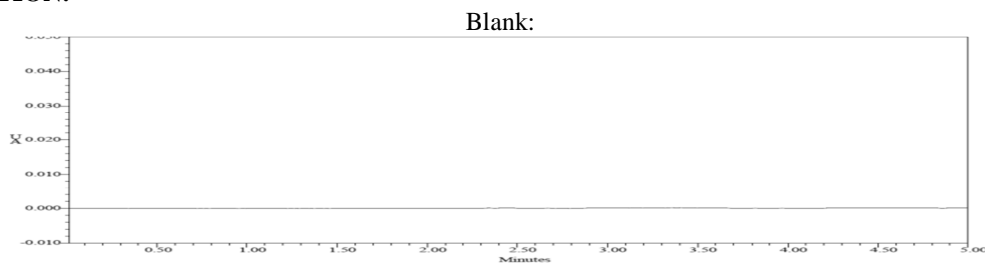


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

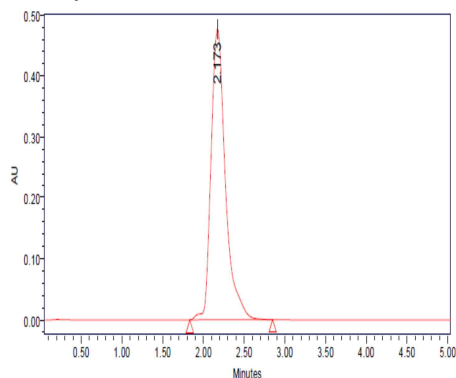


Fig: Chromatogram showing injection -1

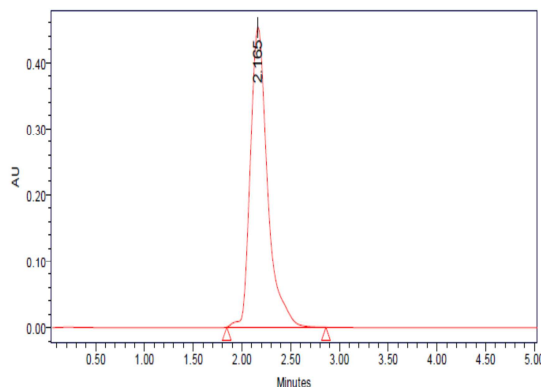


Fig: Chromatogram showing injection -2

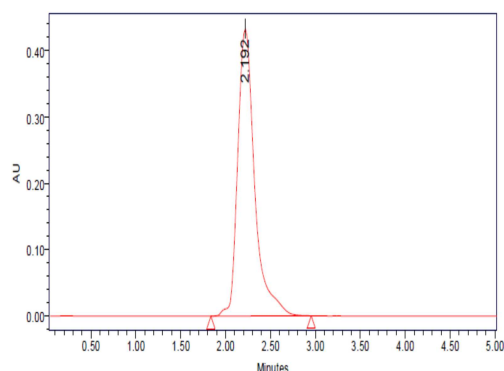


Fig: Chromatogram showing injection -3

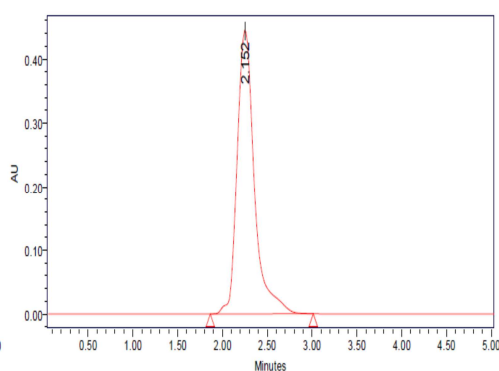


Fig: Chromatogram showing injection -4

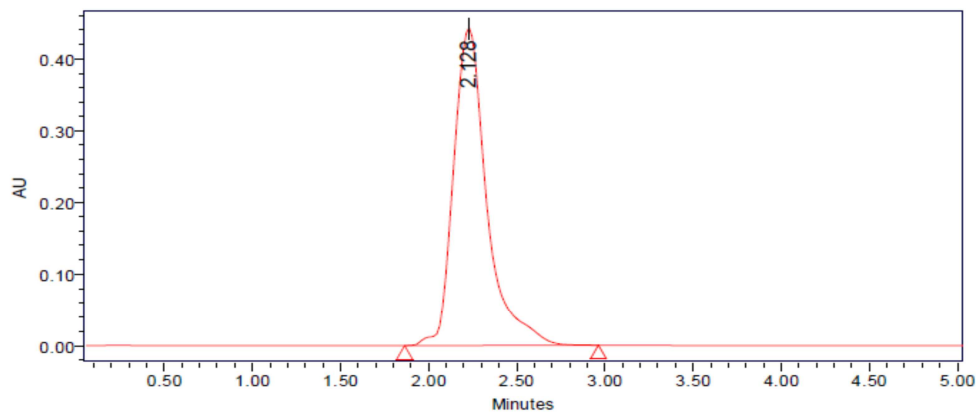


Fig: Chromatogram showing injection -5

Table: Results of system suitability for Tradozone

S. No	Peak Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Tradozone	2.173	505628	477915	5817	1.49
2	Tradozone	2.165	505894	454466	5810	1.49
3	Tradozone	2.192	506855	433328	5777	1.60
4	Tradozone	2.152	520667	430583	5759	1.53
5	Tradozone	2.128	509199	442971	5783	1.54
Mean			509648.6			
Std. Dev.			6318.103			
% RSD			1.2			

Acceptance criteria: %RSD of five different sample solutions should not more than 2, The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately Tradozone in drug product.

Assay (Standard):

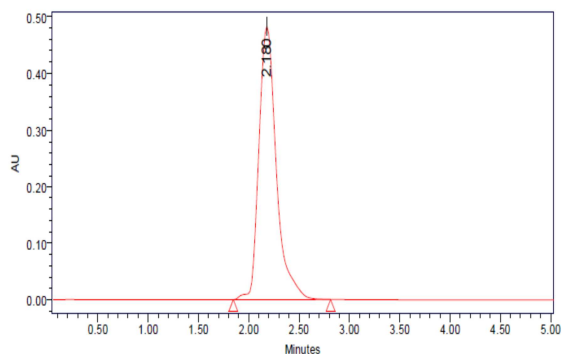


Fig. Chromatogram showing assay of Standard injection -1

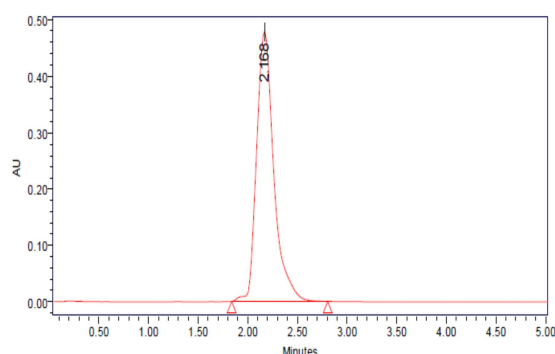


Fig. Chromatogram showing assay of standard injection -2

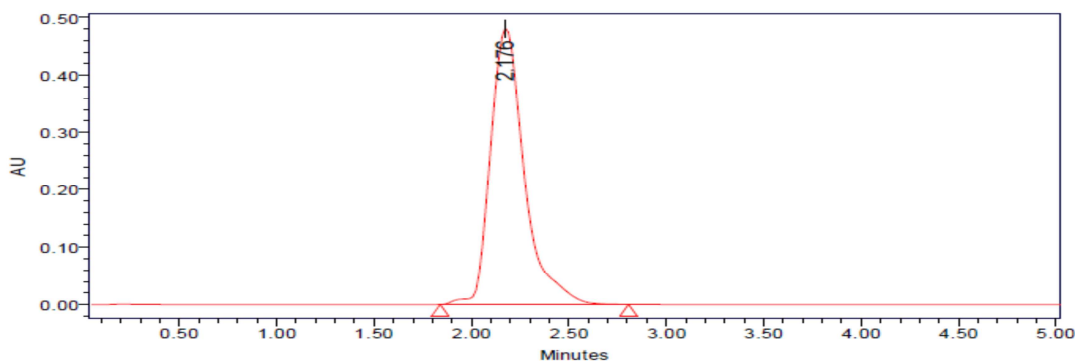


Fig. Chromatogram showing assay of standard injection -3

Table: Peak results for assay standard

Assay (Sample):

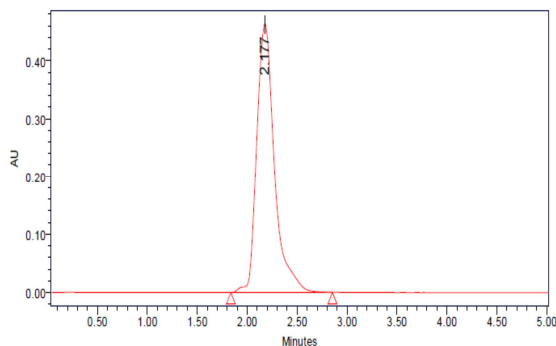


Fig: Chromatogram showing assay of sample injection-1

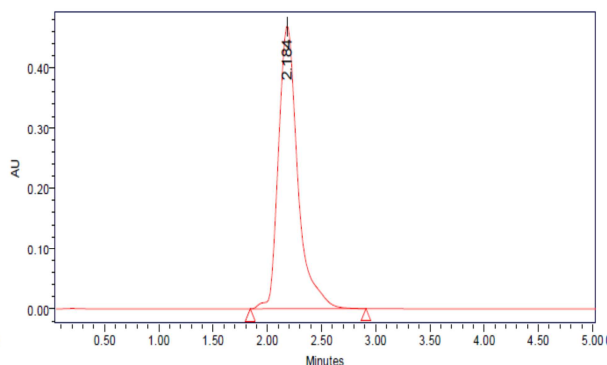


Fig: Chromatogram showing assay of sample injection-2

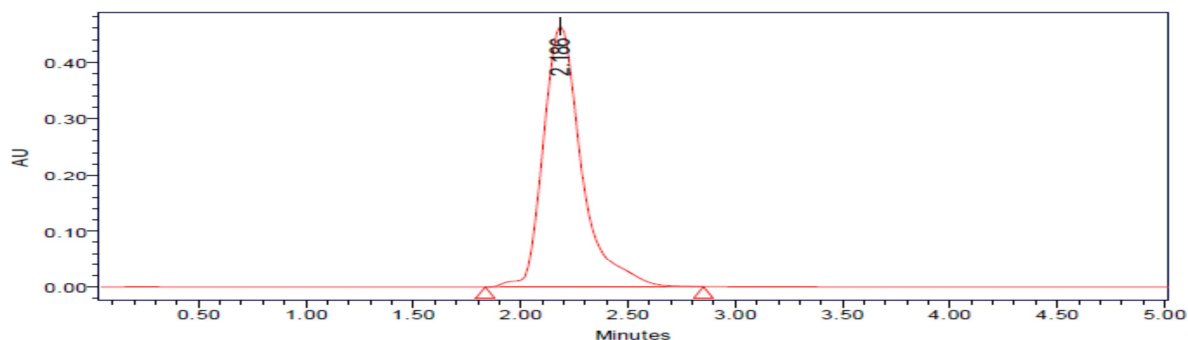
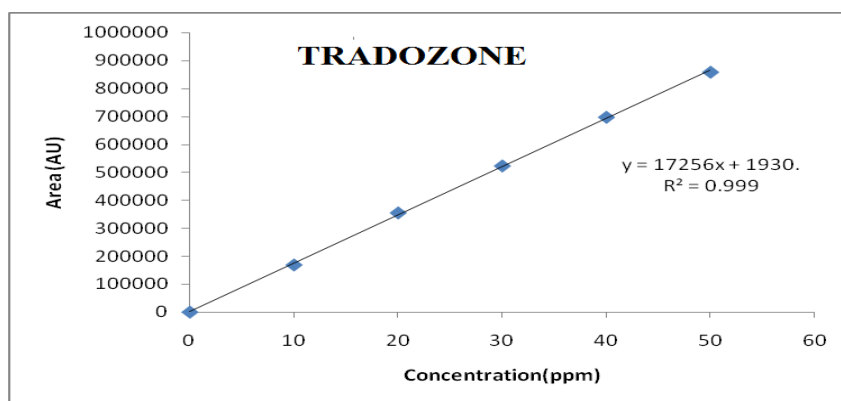


Fig: Chromatogram showing assay of sample injection-3

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Concentration Level (%)	Concentration $\mu\text{g/ml}$	Average Peak Area
33	10	168581
66	20	354709
100	30	522589
133	40	696532
166	50	857576

**LINEARITY PLOT:**

The plot of Concentration (x) versus the Average Peak Area (y) data of Tradozone is a straight line. $-Y = mx + c$, Slope (m) = 17256, Intercept (c) = 1930, Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 1930. These values meet the validation criteria.

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

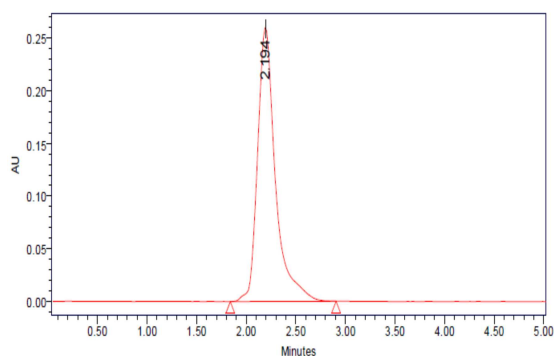
Accuracy 50%:

Fig. Chromatogram showing accuracy-50% injection-1

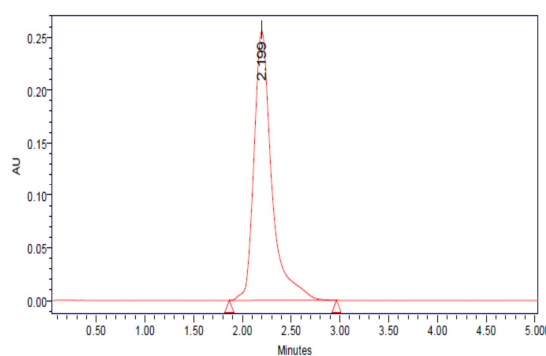


Fig. Chromatogram showing accuracy-50% injection-2

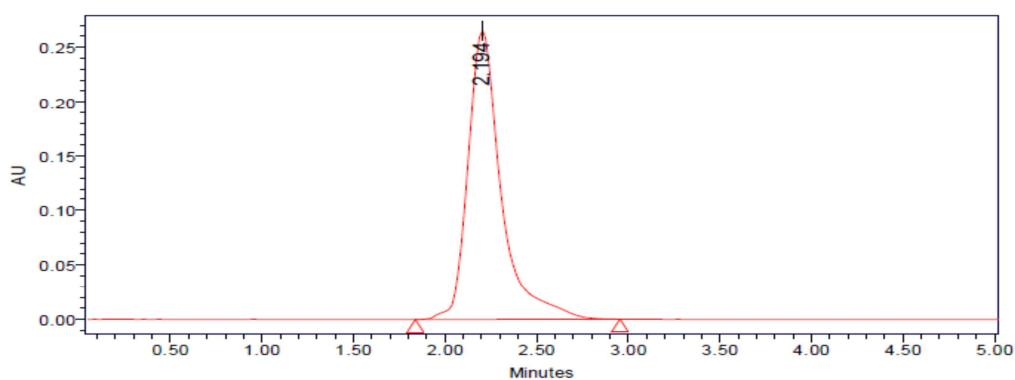


Fig. Chromatogram showing accuracy-50% injection-3

Table: Results of Accuracy for concentration-50%

S. No	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Injection
1	Tradozone	2.194	280386	260114	1.57	5841	1
2	Tradozone	2.199	256038	256856	1.65	5850	2
3	Tradozone	2.194	250487	265065	1.62	5859	3

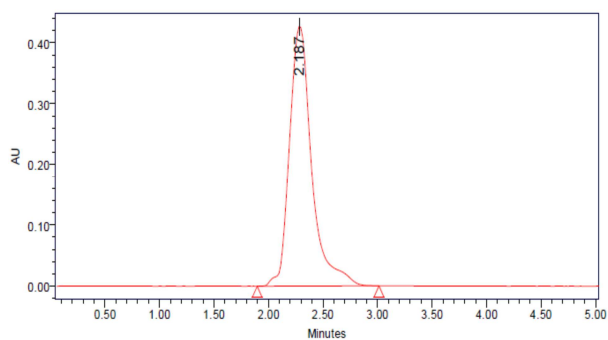
Accuracy100%:

Fig Chromatogram showing accuracy-100% injection-1

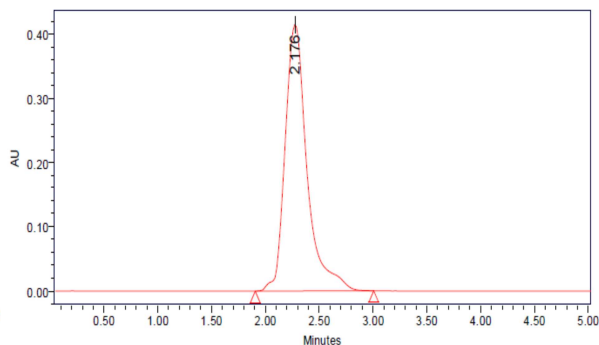


Fig Chromatogram showing accuracy-100% injection-2

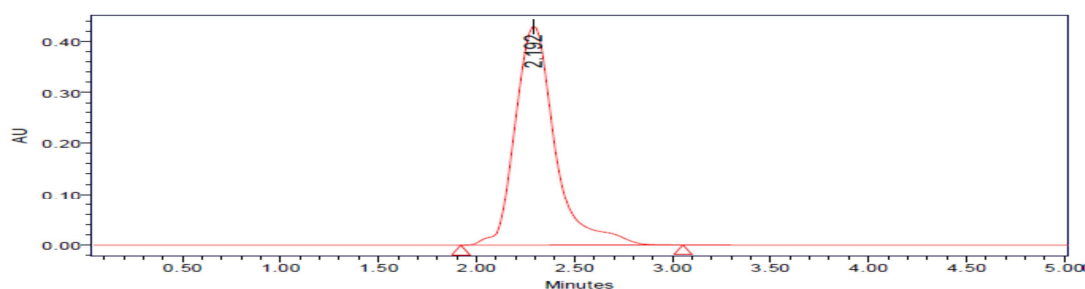


Table Results of Accuracy for concentration-100%

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Tradozone	2.176	527090	415034	1.67	5705	1
2	Tradozone	2.187	513889	427279	1.57	5719	2
3	Tradozone	2.192	509669	429669	1.54	5723	3

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Tradozone in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Tradozone was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Tradozone in bulk drug and in Pharmaceutical dosage forms.

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