An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TINIDAZOLE TABLETS RELATED SUBSTANCES BY RP-HPLC

P. Jyothi*[1], Y. Suresh Reddy[2]

[1]Department of Pharmaceutical Analysis, A.M Reddy memorial College of Pharmacy, Narasaraopet, Andhra Pradesh, India.

[2]Department of Analytical Chemistry, K. L University, Guntur, Andhra Pradesh, India.

***Corresponding author E-mail:** lathaudayan94@gmail.com

ARTICLE INFO

Key Words

RP-HPLC,

Tinidazole.

ABSTRACT

A Simple, precise and accurate method was developed for the estimation of Tinidazole and related impurities analysis. For RP-HPLC method Mobile phase is Acetonirtrile: Methanol: Water (10:20:70) in the ratio of 10:20:70 v/v was selected as a mobile phase gave retention time at 6.0 min for Tinidazole. The column used was Zorbax C-8, 250*4.6mm, 5µm (or) Equivalent with flow rate 1ml/min using UV detection at 320nm. The correlation coefficient of Tinidazole was found to be should not be less than 0.995.The limit of quantification for Tinidazole was found to be 0.2µg/ml. The accuracy was found to be within the limits. The precision was within the acceptance criteria not more than 5.0% for each individual impurity. Hence it is conclude the developed RP-HPLC method can be effectively used for estimation of Tinidazole and their related impurities from pharmaceutical dosage forms.

INTRODUCTION:

Tinidazole chemically 1- (2-ethylsulfonylethyl)-2methyl-5-nitro-imidazole. The empirical formula is C8H13N3O4S and molecular weight is 247.273 g/mol. It is a synthetic nitroimidazole derivatives used as an anti-protozoal, antibacterial agent, chemically reduced tinidazole was shown to release nitrites and cause damage to purified bacterial DNA in vitro. Additionally, the drug caused DNA base changes in bacterial cells and DNA strand breakage in mammalian cells. It absorbs rapidly and completely under fasting conditions. Oral absorption of tinidazole is found to be 100%. The objective of the present study is to develop a simple, Accurate and precise HPLC method for the validation of Tinidazole tablets related substances.

MATERIALS AND METHODS

Tinidazole and Ciprofloxacin, Related impurities materials were obtained from optimus generics limited, Mahabubnagar. Water: Milli-Q , Methonal: Ramkem, Acetonitrile :Rankem

Instruments Used: HPLC : Equipped with a photodiode array detector capable operating in the range of 190 nm to 400 nm – Waters.

METHOD DEVELOPMENT

Optimized Chromatographic Conditions

Column: Zorbax C-8 ,250*4.6mm ,5µm (or) Equivalent
Flow : 1.0mL/min
Injection Volume: 20 µL
Wavelength: UV at 320nm
Column oven Temperature: 40°C
Run time: 30 Minutes
Mobile phase: Acetonitrile: Methanol: Water (10:20:70 v/v/v)
RT: Retention time of Tinidazole is at about 6.0min

Mobile Phase Preparation: Prepare a filtered and degassed mixture of Acetonitrile: Methanol: Water: (10:20:70 v/v/v).

Diluent-1: Methanol.

Diluent-2: Mobile Phase .

Blank: Mobile Phase.

Tinidazole Impurity-A stock solution-1 preparation (500 ppm): Weigh accurately about 10.0 mg of Tinidazole Impurity –A standard and transfer in to a 20 mL volumetric flask. Add about 10mL of diluent-1, sonicate for 5 minutes to dissolve and dilute to volume with diluent-1.

Tinidazole Impurity-A stock solution-2preparation (50 ppm): From the above prepared Tinidazole Impurity-Astock solution-1, pipette out 2ml in to a 20ml volumetric flask and dilute to volume with diluent-1.

Tinidazole Impurity -B stock solution-1 preparation (500 ppm): Weigh accurately about 10.0 mg of Tinidazole Impurity –B standard and transfer in to a 20 mL volumetric flask. Add about 10mL of diluent-1, sonicate for 5 minutes to dissolve and dilute to volume with diluent-1.

Tinidazole Impurity-B stock solution-2 preparation (50 ppm): From the above prepared Tinidazole Impurity-B stock solution-1, pipette out 2ml in to a 20ml volumetric flask and dilute to volume with diluent-1.

Tinidazole stock solution-1 preparation (500 ppm): Weigh accurately about 50.0 mg of Tinidazole standard and transfer in to a 100 mL volumetric flask. Add about 60mL of diluent-1, sonicate for 5 minutes to dissolve and dilute to volume with diluent-1.

Tinidazole stock solution-2 preparation (10 ppm): From the above prepared Tinidazole stock solution-1,pipette out 1ml in to a 50ml volumetric flask and dilute to volume with diluent-1.

System suitability solution preparation: From the above prepared stock solutions, pipette out each 1ml of Tinidazole Impurity-A stock solution-2 and Tinidazole Impurity-B stock solution-2 in to a 25ml volumetric flask and dilute to volume with diluent-2.(solution having a known concentration about 2ppm of Tinidazole Impurity-A&2ppm of Tinidazole Impurity-B respectively)

Standard solution preparation (2 ppm): From the above prepared Tinidazole stock solution-2, pipette out 5ml in to a 25ml volumetric flask and dilute to volume with diluent-2.

Placebo solution preparation: Weigh accurately about 75 mg of the Tinidazole placebo and transfer in to a 50ml volumetric flask. Add about 35mL of diluent-1, sonicate for 10 minutes to dissolve and dilute to volume with diluent-1.Filter through 0.45 µ PVDF filter. Further pipette out 5ml of above filtered solution in to a 50ml volumetric flask and dilute to Volume with diluent-2.

Sample solution preparation (1000 ppm): Weigh accurately and take 20 tablets and crush as fine powder. Accurately weigh and transfer about 575mg of the tablets powder (Equivalent to about 500mg of Tinidazole)in to a 50ml volumetric flask. Add about 35mL of diluent-1, sonicate for 10 minutes to dissolve and dilute to volume with diluent-1.Filter through 0.45 µ PVDF filter. Further pipette out 5.0 mL of above filtered solution in to a 50 mL volumetric flask and diluted to volume with diluent-2.

Table: 1 Relative retentions time for impurities

Name of the compound	Relative Retention Time
Tinidazole Impurity-A	About 0.6
Tinidazole	About 1.0
Tinidazole Impurity-B	About 0.7

Retention times in Individual Standard Solution

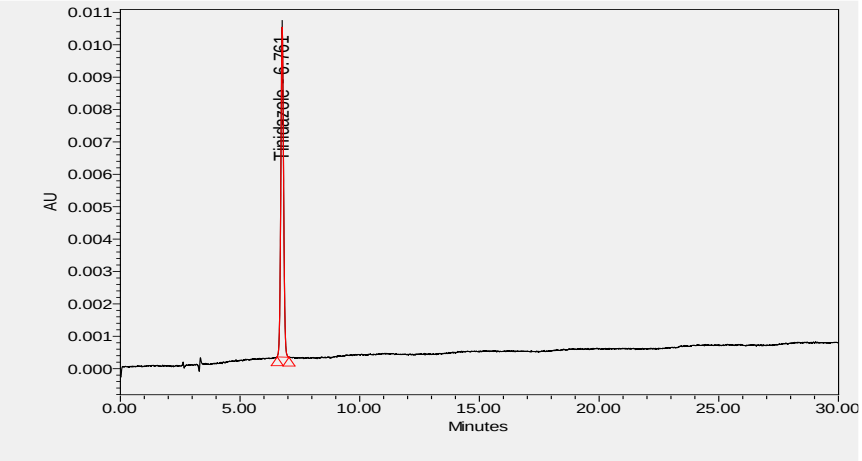

Fig.12 Chromatogram of Tinidazole

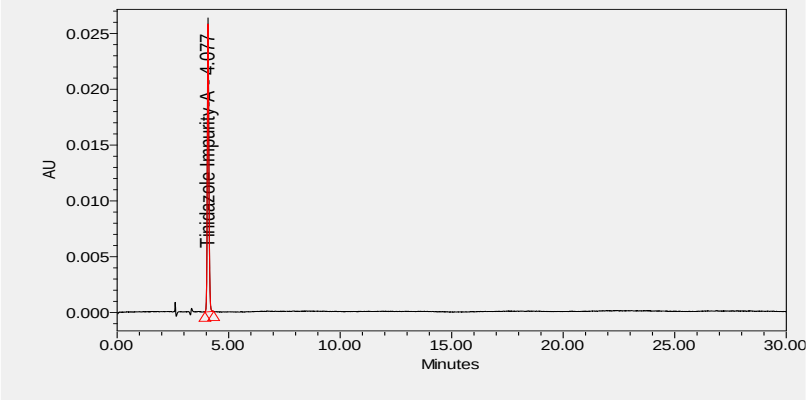

Fig.13 Chromatogram of Tinidazole impurity-A

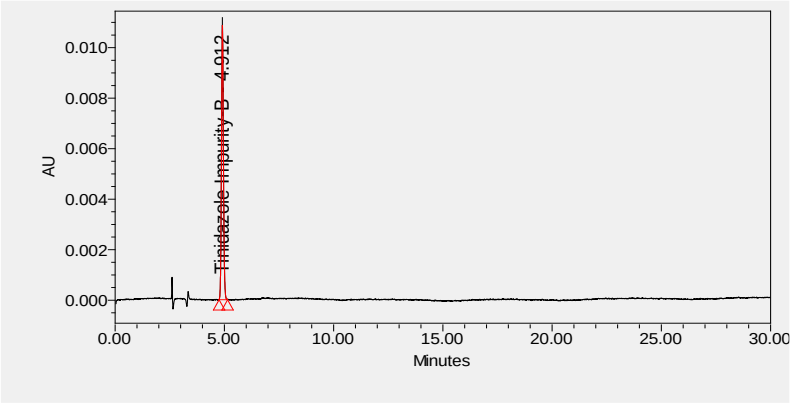

Fig.14 Chromatogram of Tinidazole impurity-B

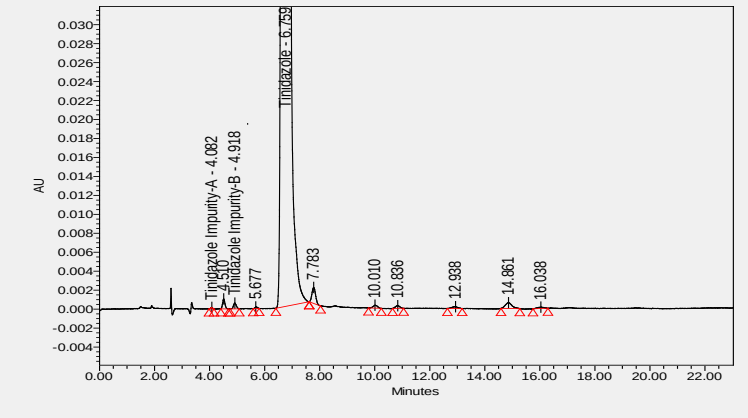

Fig:1 Chromatogram of sample solution

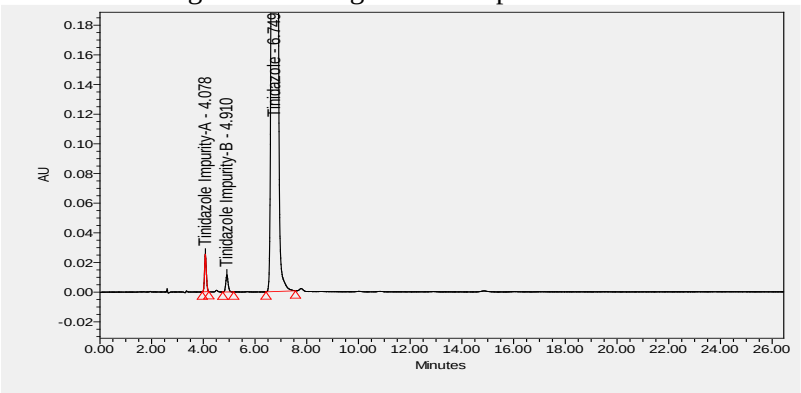

Fig:2 Chromatogram of spiked sample solution

Limit of quantification and limit of detetction

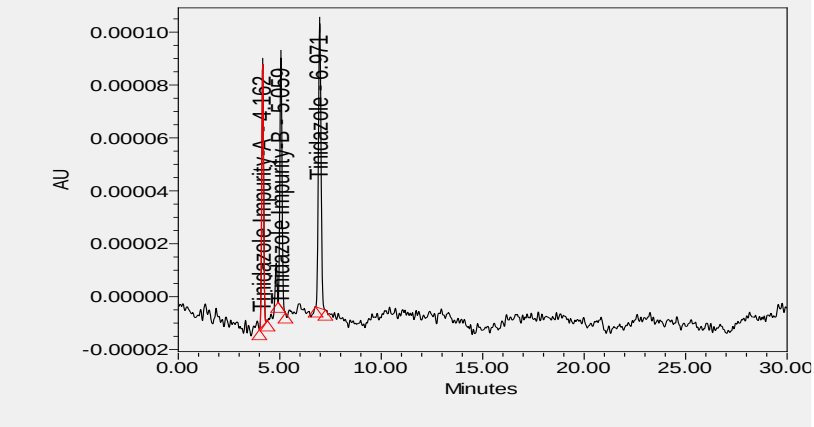

Fig:3 Chromatogram of Limit of quantification

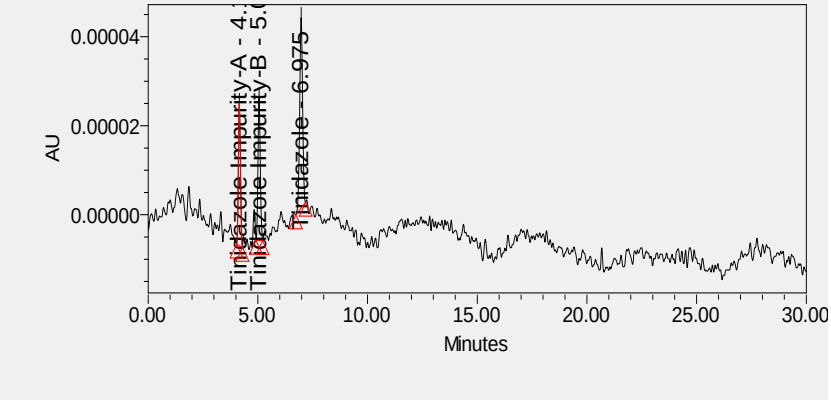

Fig:4 Chromatogram of Limit of detection

LINEARITY

Tinidazole Linearity Plot (Concentration Vs Peak area response)

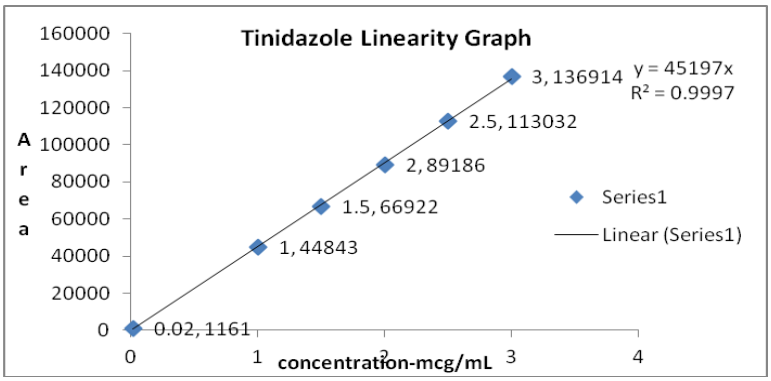

Fig.5 Tinidazole Linearity Plot

Tinidazole impurity-A Linearity Plot (Concentration Vs Peak area response)

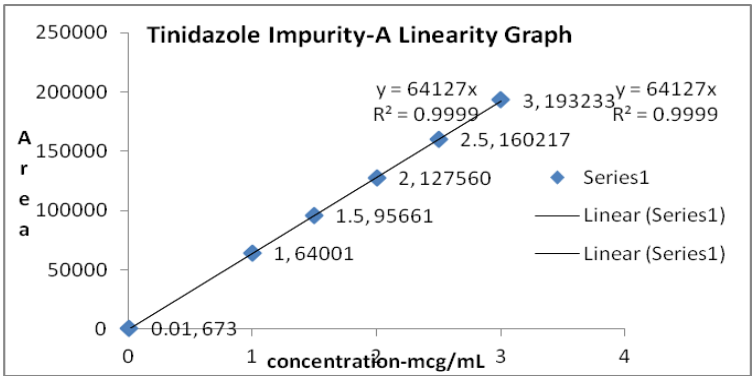

Fig.5 Tinidazole impurity-A Linearity Plot

Tinidazole impurity-B Linearity Plot (Concentration Vs Peak area response)

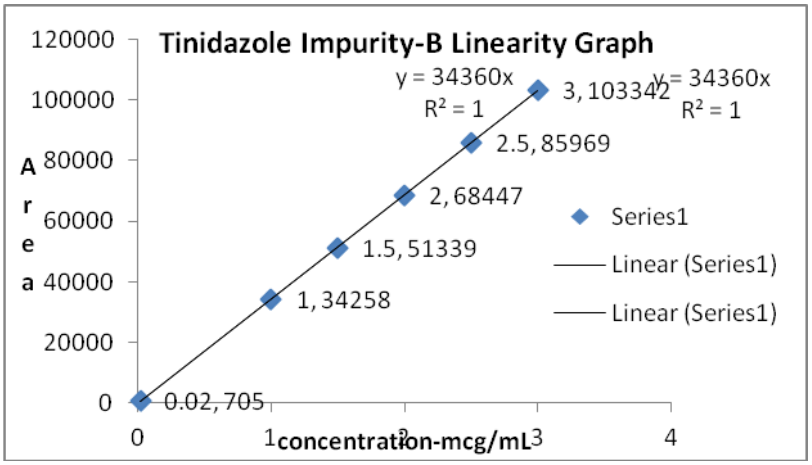

Fig.6 Tinidazole impurity-B Linearity Plot

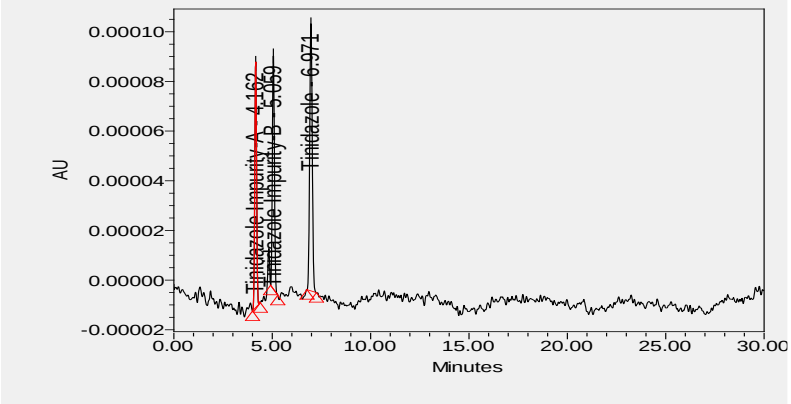

Fig.7 Chromatogram of LQQ level-1

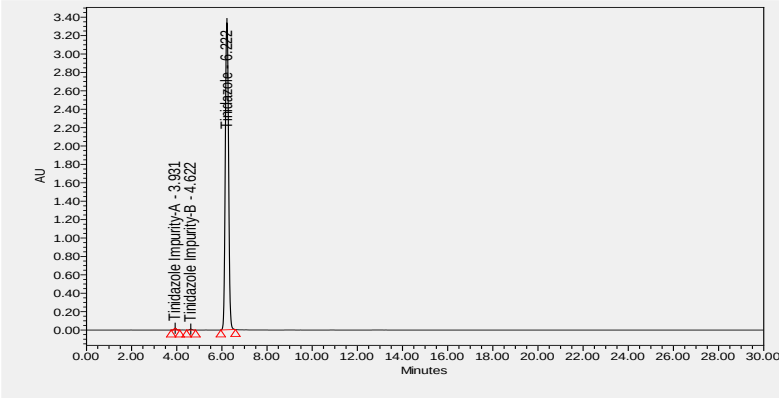

Fig.8 Chromatogram of 100% level-1

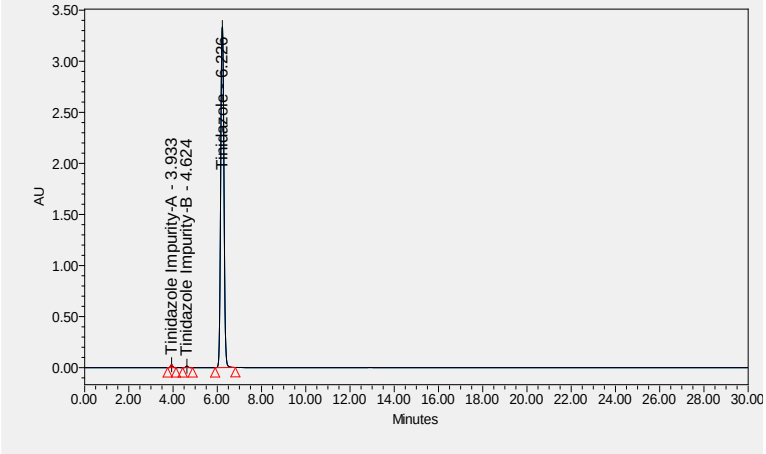

Fig.9 Chromatogram of 150% level-1

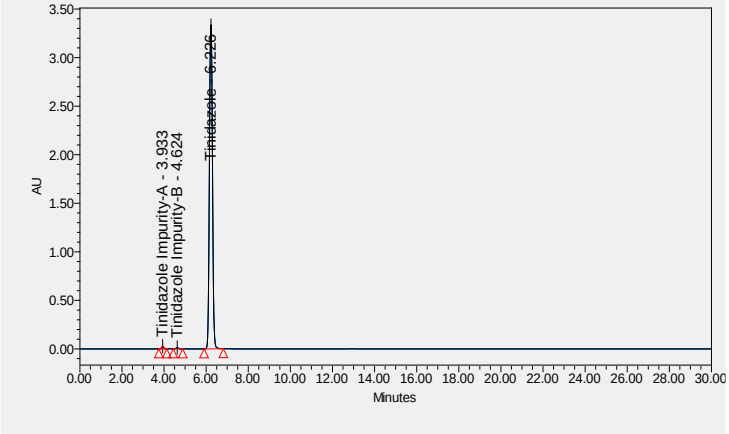

Fig.10 Chromatogram of system precision-1

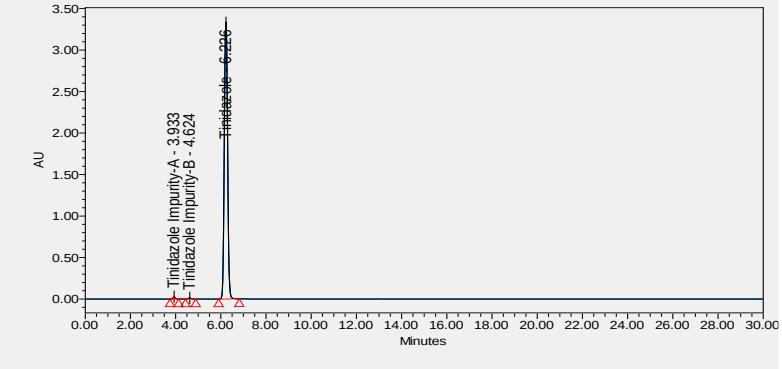

Fig.11 Chromatogram of method precision-A-1

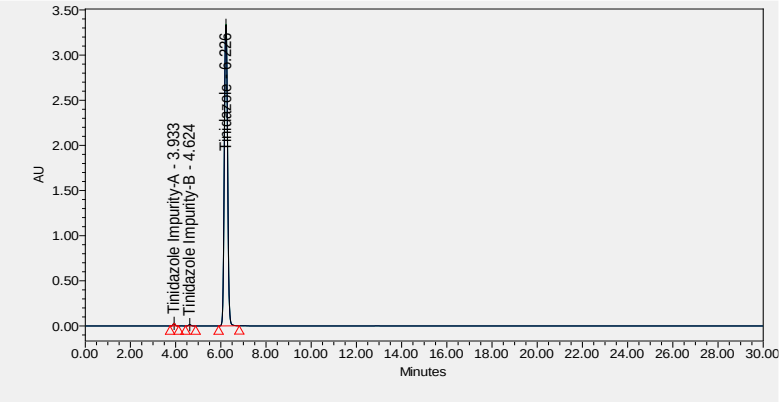

Fig.12 Chromatogram of method precision-B-1

Robustness:

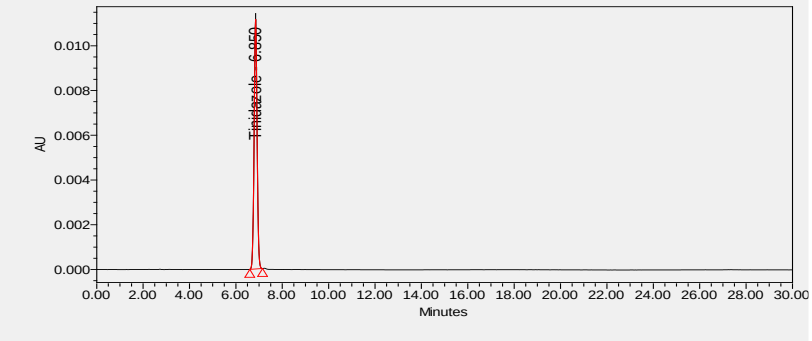

Fig.13 Chromatogram of low flow-1

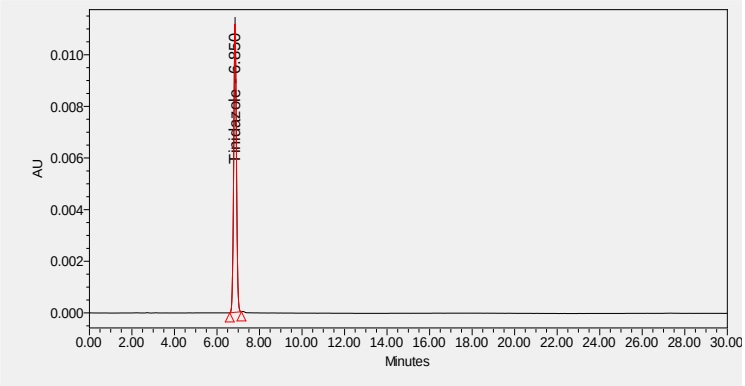

Fig.14 Chromatogram of high flow-1

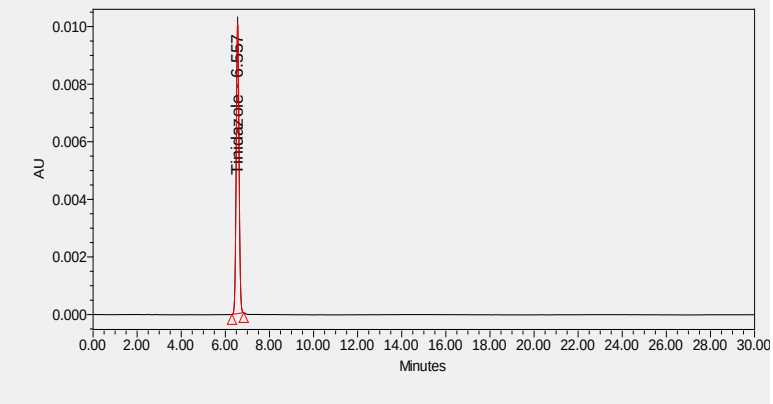

Fig.15 Chromatogram of low temp-1

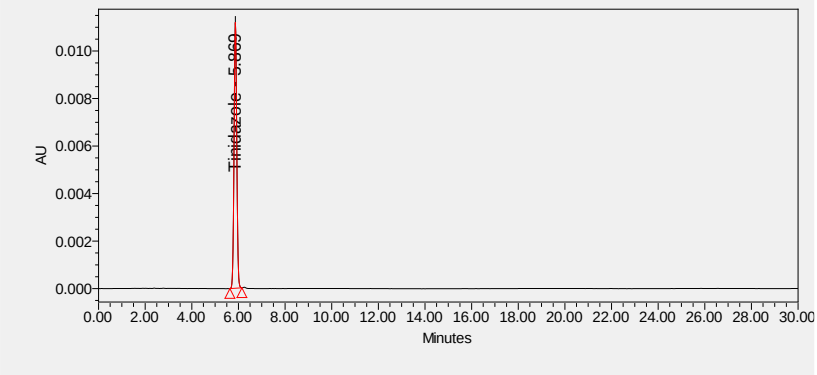

Fig.16 Chromatogram of high temp-1

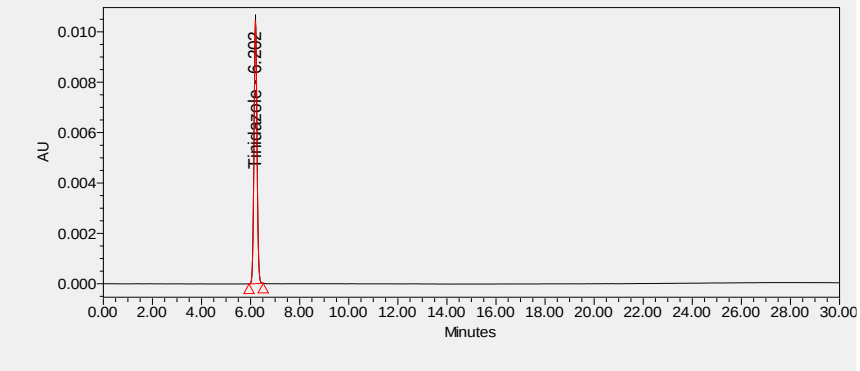

Fig.17 Chromatogram of high wave length-1

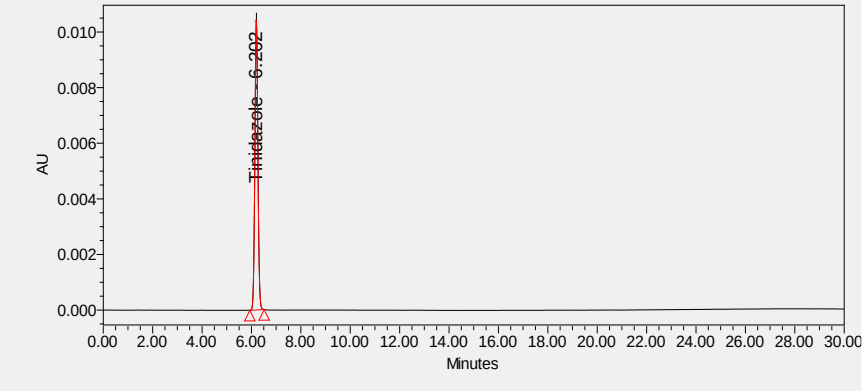

Fig.18 Chromatogram of high wave length-1

VALIDATION PARAMETERS

A.Specificity and system suitability

Identification & RT conformation: Prepared Tinidazole impurity-A, Tinidazole impurity-B, Tinidazole standard and sample solutions as per methodology and Prepared spiked sample solution at impurity specification level and injected in to the chromatographic system. Prepared Blank and placebo solution as per methodology. Injected into the chromatographic system. From the system suitability studies it was observed that all the parameters were within limit. Hence it was concluded that the Instrument, Reagents and Column were suitable to perform the Assay. Blank and placebo should not show any peak at the retention time of analyte peak

B.LIMIT OF QUANTIFICATION AND LIMIT OF DETETCTION

Limit of quantification: Established the limit of quantification Tinidazole impurity-A, Tinidazole impurity-B and Tinidazole by calculating S/N ratio at lower concentrations. Prepared LOQ level solution of Tinidazole impurity-A, Tinidazole impurity-B and Tinidazole and injected into the chromatographic system. S/N ratio should be between 10.0 to 30.0

Limit of detetction: Established the limit of detection for Tinidazole impurity-A, Tinidazole impurity-B and Tinidazole by calculating S/N ratio at lower concentrations. S/N ratio should be between 3.0 to 9.0.

Precision: Precision of the test method by injecting six test samples prepared by spiking all the impurities at specification limit to the target concentration. And also precision study for diluted standard, by spiking working standard at specification level (maximum allowable % level for unknown impurity) on placebo. Injected the solutions in the chromatographic system as per the method then calculate the individual % Relative standard deviation of diluted standard and all the impurities

C.Linearity: Performed the linearity for Tinidazole impurity-A, Tinidazole impurity-B and Tinidazole from LOQ to 150% of specification level by taking minimum five concentration levels. Injected each LOQ & 150% concentration level in six replicates and remaining concentrations in duplicate. Plotted a graph of concentration against the peak area responses and calculated the linearity regression coefficient and y- intercept. The response of tinidazole, tinidazole impurity-A and tinidazole impurity-B was linear from 0.02mcg/ml to 3.0mcg/ml, 0.01mcg/ml to 3.0mcg/ml and 0.02mcg/ml to 3.0mcg/ml of standard concentration respectively. Correlation coefficient should not be less than 0.995.

D. Accuracy: Prepared six sample solutions by spiking Tinidazole impurity-A and Tinidazole impurity-B at specification levels and inject into the chromatographic system. At concentration levels ranging from LOQ to 150% of specification level using at least three replicates of minimum three concentration levels and injected into the chromatographic system. To the placebo solution of Tinidazole tablets, Tinidazole impurity-A and Tinidazole impurity-B spiked at LOQ of sample concentration level in triplicate and injected each solution into the chromatographic system. The average % recovery of Tinidazole impurity-A and Tinidazole impurity-B was calculated. % Recovery of Tinidazole impurity-A, Tinidazole impurity-B, should be between 80.0 -120.0 at LOQ level.% Recovery of Tinidazole impurity-A and Tinidazole impurity-B should be between 85.0 -115.0 for LOQ, 100% and 150% Levels. %RSD for %recovery for each level of known impurity should be not more than 10.0%

E. Intermediate precision

System Precision: Prepared System suitability solution and Tinidazole standard solution as per methodology and injected System suitability solution single time and Tinidazole standard six times into

the chromatographic system and calculated the % RSD for area of Tinidazole in standard solution. The test result shows that the system was precise

F.Robustness

System Precision and Robustness: Prepared System suitability solution and Tinidazole standard as per methodology and inject System suitability solution single time and Tinidazole standard six replicates into the chromatographic system. at below mentioned chromatographic conditions

a. Flow rate (+ 10%)

From 1.0 mL/min to 0.9 mL/min(-Flow)

From 1.0 mL/min to 1.1 mL/min (+Flow)

b. Temperature

From 40°C to 35°C(-Temperature)

From 40°C to 45°C(+Temperature)

C. Wavelength Variation(±2nm)

From 320nm to 318nm

From 320nm to 322nm

The observed values are within the acceptance criteria for Robustness study.

G. solution stability:

Prepared the standard solution and sample solution as per methodology. Carry out the solution stability for standard solution and sample solution at bench top and refrigerator by injected at regular intervals (eg: 6Hrs, 12Hrs, 24Hrs &36 Hrs .) against to fresh standard solution. Difference of assay for standard at different intervals compared to initial should not be more than 5.0%. The samples at different intervals compared to Initial samples, the absolute % of difference of % m/m of Tinidazole impurity-A, Tinidazole impurity-B, single maximum unspecified impurity should not be more than 0.05 and the % of total impurities should not be more than 0.2. The standard and samples at different intervals compared to initial standard ,the absolute % of difference of % assay of Tinidazole and,%m/m of Tinidazole impurity-A, Tinidazole impurity-B, single maximum unspecified impurity and total impurities are within limits upto 36Hrs(solution stability).

CONCLUSION:

As per discussion in the Literature review, indicated that UV-Spectrophotometric, LC-MS, HPLC, RP-HPLC and HPTLC and combination methods have been reported for determination of Tinidazole in pharmaceutical dosage form. An extensive literature survey reveals few methods are reported for estimation of related substances of Tinidazole in tablet formulation by RPHPLC method. Most of the reported methods either do not include stability studies or are not completely validated, and they are cumbersome, time consuming. Recovery data for the study is reported .The method was found to be accurate with % recoveries for Tinidazole Impurity-A was 110% at LOQ level, 96.62 at 100% level & 94.98% at 150% level and % recoveries for Tinidazole Impurity-B was 96.67 at LOQ level,95.96 at 100% level&97.18 at 150% level in Tinidazoletablets. There was good repeatability of proposed method with % RSD of 0.12% for system precision.The result of specificity studies indicated no interference from excipients and Mobile phase. The developed method was validated according to ICH validation parameters. The percentage of recovery of Ciprofloxacin and Tinidazole was found to be 100.4, 101.9 at 100% level. their related substances was found to be with in acceptance range at 100% level. The standard deviation values and good recoveries indicate the reproducibility and accuracy of the developed method. As well the % RSD values for precision study also were within acceptable limit.

Acknowledgement: We are heartly thankful our respective director of Optimus generics limited who given permission to carry out this work and I thankful to JN Reddy who supported us.

REFERENCES:

1. ICH Q3B(R), Impurities in New Drug Product, ICH Harmonized Tripartite Guidelines, 1999.
2. Okunrobo LO. Titrimetric and Spectrophotometric Determination of Tinidazole Tablets. World Journal of Chemistry, 2(2), 2007, 63-66.
3. ICH Harmonized Tripartite Guideline ICH Q2B(R). Impurities in New Drug Product, 1999.
4. Bhatkar RG, Nagavankar CV. Spectrophotometric analysis of Tinidazole. Eastern-Pharmacist, 25, 1982, 117.
5. http://dx.doi.org/10.1155/2013/185706 Journal of analytical methods in chemistry volume 2013.article 185706,50
6. International journal of pharmacy & pharmaceutical sciences;2010 supplement 2,vol-2 issue s_2 .p46
7. http://www.academia.edu/660652/ Development and simultaneous spectrophotometric estimation of tinidazole and clotrimazole in tablet and dosage forms
8. G.A Shabir, "Validation of HPLC Chromatography Methods for Pharmaceutical analysis, Understanding the differences and similarities between validation Requirements of FDA, the US Pharmacopeia and the ICH," J.Chromatogr. A. 987(1-2), 57-66(2003)
9. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Impurities in New Drug Products Q3B(R2), current step 4 version, dated 2 June 2006
10. Principles of Instrumental Analysis, 5th Edition, Skoog, Holler and Nieman.
11. William kemp: organic spectroscopy,2008,251
12. Ravi Shankar: Text book of pharmaceutical analysis, Tirunelveli: Rx Publications, 3,2006,6-18.
13. Y.R .Sharma: Elementary organic Spectroscopy,4,2010,2.