CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com Research Article

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND TINIDAZOLE AND ITS PHARMACEUTICAL DOSAGE FORMS

G. Sindhu a* , Dr. G. Sai kiran, dr. D. Venkata Ramana.
Department Of Pharmaceutical Analysis, Holy Mary Institute Of Technology And Science (College Of Pharmacy), Keesara - Bogaram - Ghatkesar Rd, Kondapur, Telangana 501301.

Abstract:
A simple, accurate, and robust Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Ciprofloxacin and Tinidazole in combined pharmaceutical dosage forms. The chromatographic separation was achieved on a Develosil C18 column (4.6 mm × 250 mm, 5 µm particle size) using a mobile phase consisting of Acetonitrile and Acetate buffer (pH 4.3) in the ratio of 35:65% v/v, at a flow rate of 1.0 ml/min. The detection was carried out at a wavelength of 238 nm using a Waters Alliance 2695 HPLC system equipped with a PDA Detector (996 model). The injection volume was 20 µl, and the run time was 6 minutes under ambient temperature conditions. The method was validated according to ICH Q2(R1) guidelines for various parameters including specificity, linearity, accuracy, precision, LOD, LOQ, robustness, and system suitability. The results demonstrated high resolution and selectivity between Ciprofloxacin and Tinidazole with no interference from excipients. The method showed excellent linearity and recovery within acceptable limits.

Keywords: *RP-HPLC, Ciprofloxacin, Tinidazole, Develosil C18 column, linearity, accuracy.*

Corresponding author:
G. Sindhu *
Department of Pharmaceutical Analysis,
Holy Mary Institute of Technology and Science (College of Pharmacy),
Keesara - Bogaram - Ghatkesar, Telangana.
Email Id- sindhureddy7997@gmail.com

QR CODE

Please cite this article in press G. Sindhu et al., ***Rp-Hplc Analytical Method Development And Validation For Simultaneous Estimation Of Ciprofloxacin And Tinidazole And Its Pharmaceutical Dosage Forms*** *, Indo Am. J. P. Sci, 2025; 12(10).*

1. INTRODUCTION:

Chromatography is a widely used laboratory technique for separating and analyzing components of a mixture based on their differential interactions with a stationary phase and a mobile phase. It is crucial in many fields such as chemistry, biochemistry, pharmaceuticals, and environmental science for purifying, identifying, and quantifying compounds.

The term "chromatography" was coined by the Russian scientist Mikhail Tsvet in 1903, when he developed the technique to separate plant pigments. The process involves a sample being passed through a medium (stationary phase), where the different components of the sample are separated based on their varying affinities for the stationary phase and their solubility in the mobile phase.

Types of Chromatography:

1. **Paper Chromatography**: A simple technique where a liquid sample is separated on a paper strip, typically used for small-scale analysis.
2. **Thin-Layer Chromatography (TLC):** A more advanced technique that uses a thin layer of adsorbent material (like silica gel) on a plate to separate compounds.
3. **Gas Chromatography (GC):** Involves a gaseous mobile phase and is used primarily for separating volatile compounds.
4. **Liquid Chromatography (LC):** Uses a liquid mobile phase to separate components and is often employed in high-performance liquid chromatography (HPLC).
5. **High-Performance Liquid Chromatography (HPLC):** A more sophisticated form of liquid chromatography that offers higher resolution and is commonly used for the analysis of pharmaceuticals, biochemical substances, and environmental samples.
6. **Ion-Exchange Chromatography**: Separates ions and polar molecules based on their charge and affinity for the stationary phase.
7. **Size-Exclusion Chromatography (SEC):** Separates components based on their size, with larger molecules eluting faster than smaller ones.

Principle of Chromatography:

The separation in chromatography occurs due to differences in the distribution of sample components between the stationary phase (often a solid or liquid) and the mobile phase (gas or liquid). The components of the sample travel at different rates, allowing them to be isolated from each other. The rate at which a compound moves through the system depends on its chemical affinity for the stationary phase, its size, and its solubility in the mobile phase.

Applications of Chromatography:

- Purification of compounds: Isolating pure substances from mixtures.
- Identification and quantification: Analyzing complex mixtures for identification and concentration of components.
- Pharmaceutical analysis: Monitoring drug formulations, detecting impurities, and ensuring quality control.
- Environmental analysis: Detecting pollutants in air, water, and soil.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product.[1] The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behaviour of different components, elution at different time takes place. [2] The sample compound with the greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compounds with less affinity which travel faster and for a longer distance. [3] The High-Performance Liquid Chromatography is more versatile than gas chromatography since

Classification of HPLC can be done as:

- preparative HPLC and analytical HPLC (based on scale of operation)
- affinity chromatography, adsorption chromatography, size exclusion chromatography, ion exchange chromatography, chiral phase chromatography (based on principle of separation)
- gradient separation and isocratic separation, (based on elution technique)
- normal phase chromatography and reverse phase chromatography (based on modes of operation).[6]

EXPERIMENTAL METHODS

INSTRUMENTS USED

HPLC WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.

2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil

CHEMICALS USED:

Table-: Chemicals used

1	Ciprofloxacin	Provided by Sura Pharma labs
2	Tinidazole	Provided by Sura Pharma labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

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

Further pipette 0.2ml of Ciprofloxacin and 0.6ml of Tinidazole from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

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.

7. RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)
Column : Develosil C18 (4.6mm×250mm) 5µm particle size Column
Column temperature : Ambient
Wavelength : 238nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 6minutes

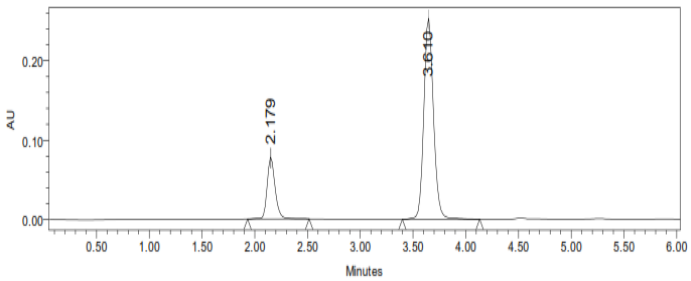

Figure-: Optimized Chromatogram (Standard)

Table-: Optimized Chromatogram (Standard)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate	Resolution
1	Ciprofloxacin	2.179	513567	78659	1.2	4536	
2	Tinidazole	3.610	1625892	265321	1.1	7985	9.8

Observation: From the above chromatogram it was observed that the Ciprofloxacin and Tinidazole peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram

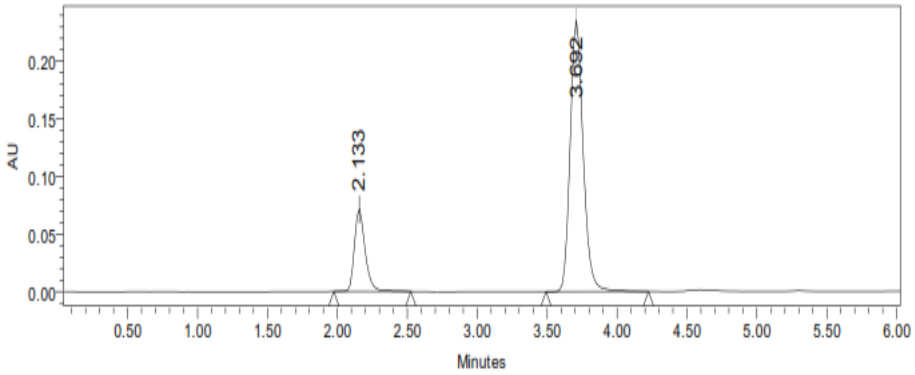

Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S.No	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Ciprofloxacin	2.133	512659	78956	1.2	4652	
2	Tinidazole	3.692	1615985	263587	1.1	7982	10.3

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.

METHOD VALIDATION

Blank:

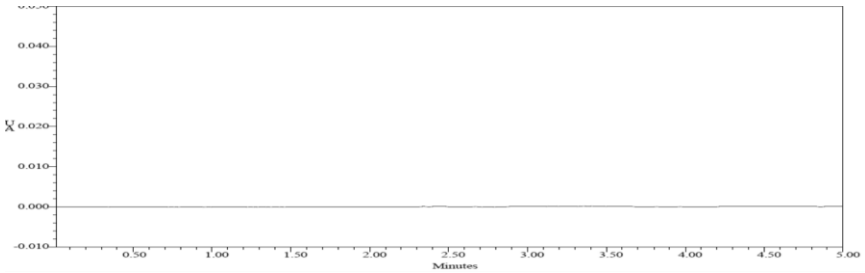

Fig-: Chromatogram showing blank (mobile phase preparation)

System Suitability:

Table-: Results of system suitability for Ciprofloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ciprofloxacin	2.152	513652	78542	4698	1.2
2	Ciprofloxacin	2.157	513524	78654	4785	1.2
3	Ciprofloxacin	2.141	513425	78541	4682	1.2
4	Ciprofloxacin	2.133	513647	78454	4854	1.2
5	Ciprofloxacin	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std. Dev.			572.2004			
% RSD			0.111363			

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.

Table-: Results of system suitability for Tinidazole

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Tinidazole	3.674	1635285	265421	7985	1.1	10.1
2	Tinidazole	3.631	1635241	265484	7898	1.1	10.1
3	Tinidazole	3.625	1652547	253498	7954	1.1	10.1
4	Tinidazole	3.692	1658458	265241	7965	1.1	10.1
5	Tinidazole	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std. Dev.			10865.58				
% RSD			0.659766				

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 quantitated Ciprofloxacin and Tinidazolein drug product.

Assay (Standard):

Table-: Peak results for assay standard of Ciprofloxacin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Ciprofloxacin	2.152	513538	78074	1.2	4562	1
2	Ciprofloxacin	2.198	513975	79001	1.2	4620	2
3	Ciprofloxacin	2.179	513283	78048	1.2	4652	3

Table-: Peak results for assay standard of Tinidazole

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Tinidazole	3.646	1625632	265325	1.1	7949	1
2	Tinidazole	3.604	1635458	265423	1.1	7919	2
3	Tinidazole	3.610	1635241	265874	1.1	7926	3

Assay (Sample):

Table-: Peak results for Assay sample of Ciprofloxacin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection	% of Assay
1	Ciprofloxacin	2.152	513265	78548	1.2	4582	1	100.1
2	Ciprofloxacin	2.150	513254	78547	1.2	4658	2	100.1
3	Ciprofloxacin	2.187	513876	78498	1.2	4597	3	99.9

Table-: Peak results for Assay sample of Tinidazole

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection	% of Assay
1	Tinidazole	3.646	1625284	78569	1.1	7985	1	100.0
2	Tinidazole	3.651	1624613	78547	1.1	7898	2	100.7
3	Tinidazole	3.601	1625874	78462	1.1	7854	3	100.6

Table: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Ciprofloxacin	0.5mg	0.4	99.57%
2	Tinidazole	10 mg	9.8	99.67%

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample Purity}}{\text{Weight of sample 100}} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Ciprofloxacin and Tinidazolein pharmaceutical dosage form was found to be 99.57% and 99.67%

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF CIPROFLOXACIN :

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

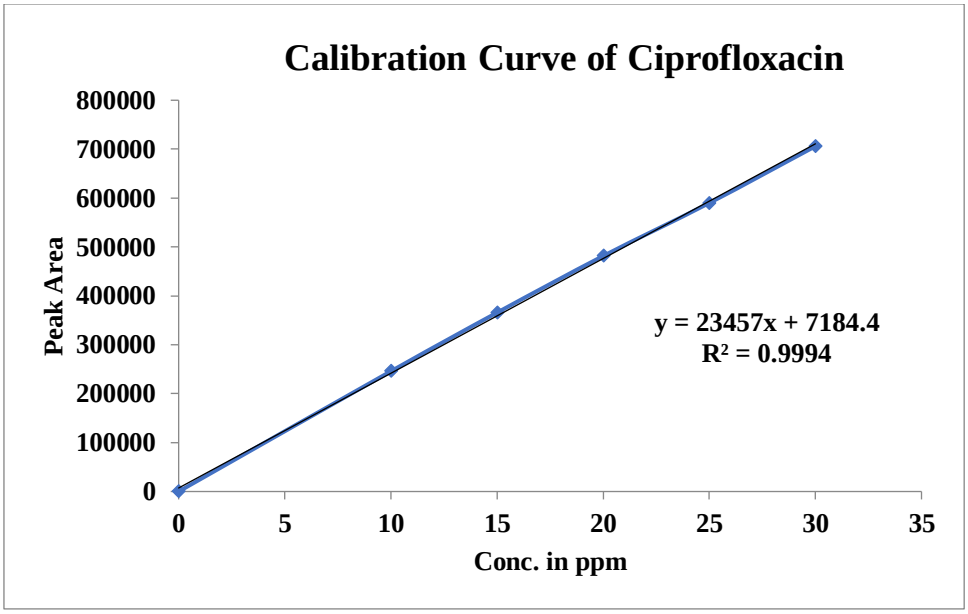

Fig-: Calibration Graph of Ciprofloxacin

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Ciprofloxacin is a straight line.

Y = mx + c

Slope (m) = 23457

Intercept (c) = 7184.4

Correlation Coefficient (r) = 0.999

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 7184. These values meet the validation criteria.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF TINIDAZOLE:

Concentration µg/ml	Average Peak Area
30	863094
45	1249397
60	1678592
75	2050412
90	2468444

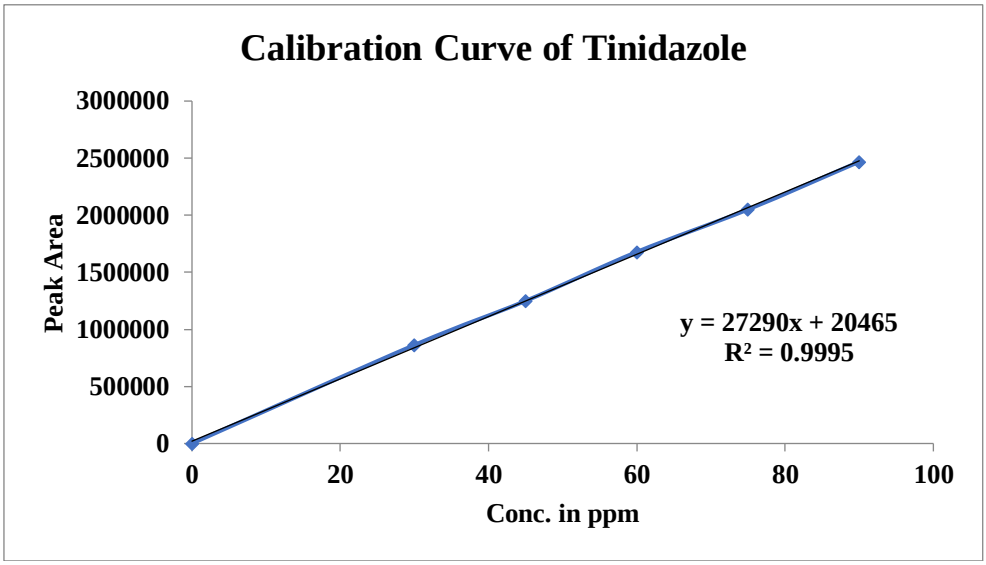

Fig-: Calibration Curve of Tinidazole

LINEARITY PLOT:
The plot of Concentration (x) versus the Average Peak Area (y) data of Tinidazoleis a straight line.

Y = mx + c
Slope (m) = 27290
Intercept (c) = 20465
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 20465. These values meet the validation criteria.

Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY
Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-: Results of repeatability for Ciprofloxacin :

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ciprofloxacin	2.157	513568	78546	1.2	4528
2	Ciprofloxacin	2.159	513685	78541	1.2	4572
3	Ciprofloxacin	2.186	513659	79852	1.2	4598
4	Ciprofloxacin	2.160	513254	78498	1.3	4529
5	Ciprofloxacin	2.170	513647	77898	1.2	4572
Mean			513562.6			

Std.dev			177.9475			
%RSD			0.03465			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise

Table-: Results of repeatability for Tinidazole:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tinidazole	3.603	1635625	265325	1.1	7985
2	Tinidazole	3.608	1658744	264588	1.1	7859
3	Tinidazole	3.600	1652985	265985	1.2	7845
4	Tinidazole	3.696	1645898	264898	1.1	7969
5	Tinidazole	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precision:

Day 1:

Table-: Results of Intermediate precision for Ciprofloxacin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ciprofloxacin	2.198	514658	78698	4658	1.2
2	Ciprofloxacin	2.196	514354	78599	4598	1.2
3	Ciprofloxacin	2.160	513985	79854	4652	1.2
4	Ciprofloxacin	2.160	514875	79879	4561	1.2
5	Ciprofloxacin	2.160	514658	79865	4659	1.2
6	Ciprofloxacin	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std. Dev.			852.3705			
% RSD			0.165563			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Tinidazole

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Tinidazole	3.623	1645875	266589	7985	1.1	10.1
2	Tinidazole	3.611	1658554	265898	8001	1.1	10.1
3	Tinidazole	3.696	1649854	265415	7985	1.1	10.1
4	Tinidazole	3.696	1659842	265154	7956	1.1	10.1
5	Tinidazole	3.696	1645985	266598	7985	1.1	10.1
6	Tinidazole	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std. Dev.			6838.733				
% RSD			0.413635				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Day 2:

Table-: Results of Intermediate precision Day 2 for Ciprofloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	US Plate count	USP Tailing
1	Ciprofloxacin	2.198	514658	78572	4672	1.2
2	Ciprofloxacin	2.196	514895	78516	4639	1.2
3	Ciprofloxacin	2.178	514658	78572	4783	1.2
4	Ciprofloxacin	2.142	514784	78372	4623	1.2
5	Ciprofloxacin	2.177	515268	78592	4639	1.2
6	Ciprofloxacin	2.177	514598	78526	4737	1.2
Mean			514810.2			
Std. Dev.			248.5224			
% RSD			0.048275			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Tinidazole

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Tinidazole	3.611	1638732	264384	7985	1.1	10.1
2	Tinidazole	3.623	1637438	265827	7946	1.1	10.1
3	Tinidazole	3.684	1638474	266382	7943	1.1	10.1
4	Tinidazole	3.697	1634273	269183	7964	1.1	10.1
5	Tinidazole	3.684	1636372	261931	7968	1.1	10.1
6	Tinidazole	3.684	1639283	264356	7982	1.1	10.1
Mean			1637429				
Std. Dev.			1860.366				
% RSD			0.113615				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

ACCURACY:

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

Table-: The accuracy results for Ciprofloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	

150%	715961	30	30.	100.72%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

Table-: The accuracy results for Tinidazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

CIPROFLOXACIN

Result:

= 1.0µg/ml

TINIDAZOLE

Result:

= 11.0µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

CIPROFLOXACIN

Result:

=3.1µg/ml

TINIDAZOLE

Result:

=3.2µg/ml

ROBUSTNESS

Table-: Results for Robustness

CIPROFLOXACIN

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.179	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0

Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

TINIDAZOLE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.610	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

8. SUMMARY AND CONCLUSION:

A precise and reliable RP-HPLC method was successfully developed and validated for the simultaneous estimation of Ciprofloxacin and Tinidazole in pharmaceutical dosage forms. Chromatographic separation was achieved using a Develosil C18 column (4.6 mm × 250 mm, 5 µm particle size) under ambient temperature conditions. The mobile phase consisted of Acetonitrile and Acetate buffer (pH 4.3) in the ratio of 35:65% v/v, delivered at a flow rate of 1.0 ml/min. Detection was carried out at a wavelength of 238 nm using a Waters Alliance 2695 HPLC system equipped with a PDA Detector (996 model). The injection volume was 20 µl, and the total run time was 6 minutes.

The method was validated in accordance with ICH Q2(R1) guidelines, evaluating parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and system suitability. The method demonstrated excellent resolution between Ciprofloxacin and Tinidazole, with sharp, well-defined peaks and no interference from excipients or other formulation components. Linearity was observed across suitable concentration ranges, and recovery studies confirmed the method's accuracy. Precision and robustness studies further established the reliability of the method under varied analytical conditions.

CONCLUSION:

The developed RP-HPLC method is simple, accurate, specific, and robust, making it suitable for the simultaneous estimation of Ciprofloxacin and Tinidazole in bulk and pharmaceutical dosage forms. The method complies with ICH validation parameters and can be confidently applied for routine quality control, assay analysis, and stability testing in pharmaceutical industries. The optimized chromatographic conditions allow for efficient separation within a short run time, making this method cost-effective and time-saving for regular analytical use.

ACKNOWLEDGEMENT
The Authors are thankful to the Management and Principal, Holy Mary Institute of Technology and Science (College of Pharmacy), Keesara - Bogaram - Ghatkesar, Telangana, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Pharma Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

9. BIBLIOGRAPHY:

1. Rao BV,Sowjanya GN,Ajitha A, Rao Uma MV. A review on stability indicating HPLC method development,World journal of pharmacy and pharmaceutical sciences.2015; 4(8):405-423.
2. Rajan HV. Development and validation of HPLC method - A Review.International Journal of current research in pharmacy. 2015;1(2):55-68.

3. 3. Kumar V, Bharadwaj R, Gupta G, Kumar S. An Overview on HPLC Method Development, Optimization and Validation process for drug analysis. The Pharmaceutical and Chemical Journal. 2015; 2(2):30-40.
4. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(4):17-25.
5. Sonia K, Nappinnai M. Development and validation of HPLC and UV-visible spectrophotometric method for the pharmaceutical dosage form and biological fluid –review. European Journal of Biomedical and Pharmaceutical sciences. 2016; 3(3): 382-391.
6. Sánchez MLF. Chromatographic techniques, European RTN Project, GLADNET, retrieved on 05-09-2013.
7. Snyder LR, Kirkland JJ, Glach JL. Practical HPLC Method Development, John Wiley and Sons, New York, 1997; 158-192.
8. HPLC – Chemiguide. May 2, 2007. www.chemguide.co.uk
9. Rao G, Goyal A. An Overview on Analytical Method Development and Validation by Using HPLC. The Pharmaceutical and Chemical Journal, 2016; 3(2): 280-289.
10. McpolinOona.an Introduction to HPLC for Pharmaceutical Analysis. Mourne Training Service. 11-12.
11. http://www.scribd.com/doc/9508765/Physical-Properties-of-Drug.
12. Buffers and pH Buffers: available from: www.xtremepapers.com.
13. Charde MS, Welankiwar AS and Kumar J. Method development by liquid chromatography with validation.International Journal of Pharmaceutical Chemistry.2014; 4(2):57-61.
14. Ranjit singh. HPLC method development and validation. J Pharm Educ Res2013; 4(1): 26-33.
15. Sabir AM, Molony M,Parminder SB. HPLC Method Development and validation: A Review. International research Journal of pharmacy. 2013; 4(4):39-46.
16. Noman A, Bukhaiti ALWedad Q, Alfarga A,AbedSherif M, Mahdi AA. And Waleed AA. HPLC technique used in food analysis-Review. International Journal of Agriculture Innovations and Research. 2016; 5(2):181-188.
17. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons. New York. 2011.
18. Xiang Y, Liu Y, Lee ML. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography. 2006; 1104(1): 198-202.
19. Horvath CG, Preiss BA, Lipsky SR. Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers. Analytical chemistry, 1967; 39(12): 1422-1428.
20. Malviya R, Bansal V, Palo P, and Sharma PK. High Performance Liquid Chromatography: A Short Review. Journal of Global Pharma Technology. 2010; 2(5):22-23.
21. Pratap B. et al. Importance of RP-HPLC in Analytical method development: A review. International journal of novel trends in pharmaceutical sciences 2013; 3(1): 15-23.
22. Lindholm J. Development and Validation of HPLC method for Analytical and Preparative Purpose. Acta Universities Upsaliensis Uppsala. 2004; 13-14.
23. Snyder LR, Kirkland JJ, Glach JL. Practical HPLC Method Development, 2nd edition. New York. John Wiley &Sons. 1997; 233-291.
24. Sethi PD. Introduction – High Performance Liquid Chromatography, 1st edn, CBS Publishers, New Delhi. 2001; 1-28.
25. Belal F et.al. Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. J Chromat Separation Techniq. 2013; 4(1): 1-7.
26. Chetta N. et.al. Development and validation of a stability indicating high performance liquid chromatographic (HPLC) method for Atenolol and hydrochlorothiazide in bulk drug and tablet formulation. Int J Chem tech res. 2013; 1(3): 654-662.
27. Kumar GS. et.al. Development and validation of RP-HPLC method for simultaneous estimation of Atenolol and Chlorthalidone in Bulk and dosage form. Int Res J Pharma 2013; 3(10): 215-19.
28. FDA Guidance for Industry (2000)-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER).
29. Julia T, Mena AJ, Aucoin MG, Kamen AA. Development and validation of a HPLC method for the quantification of baculovirus particles. J Chromatogr B. 2011; 879: 61-68.
30. Santhosh G, Nagasowjanya G, Ajitha A, Uma Maheswara Rao Y. HPLC method development and validation: an overview. International Journal of Pharmaceutical Research & Analysis. 2014; 4(2): 274-280.
31. Kayode J, Adebayo. Effective HPLC method development. Journal of Health, Medicine and Nursing.2015; 12: 123-133.
32. Gad S. Pharmaceutical manufacturing handbook of regulations and quality. John wiley and sons; 2006.
33. Webster P. Analytical procedures and method validation. Enviormental protection agency; 2001.

34. Mohamad T, Mohamad MA, Chattopadhyay M. Particle size role, Importance and Strategy of HPLC Analysis An update. International Archives of BioMedical and Clinical Research. 2016; 2(2): 5-11.
35. Weston A, Brown PR. HPLC and CE Principles and Practice. Academic press California; 1997.
36. Ngwa G. Forced Degradation Studies. Forced Degradation as an Integral part of HPLC Stability Indicating Method Development. Drug Delivery Technology. 2010; 10(5).
37. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Mott MG. Available Guidance and Best Practices for Conducting Forced Degradation Studies. Pharmaceutical Technology. 2002; 48-56.
38. Shah RS, Pawar RB, Gayakar PP. An analytical method development of HPLC.International Journal of Institutional Pharmacy and Life Sciences. 2015; 5(5): 506-513.
39. ICH Q2 (R1) Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, IFPMA, Geneva; 2005.
40. ICH Q2A. Text on Validation of Analytical Procedures, International Conference on Harmonization. Geneva; 1994.