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Research

RP-HPLC Method and its Validation for Analysis of Ofloxacin and Satranidazole in Bulk and Pharmaceutical Dosage Form

Sai Shivani*, Dr. V. Jagadeesh Babu, Dr. D. Varun

Department of Pharmaceutical Analysis, Sri Indu Institute of Pharmacy, Sheriguda (V), Ibrahimpatnam, Telangana, 501510

*Author for Correspondence: Sai Shivani
Email: nareshpamojuios@gmail.com

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Abstract

A simple, precise, and accurate Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Ofloxacin and Satranidazole in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm) with a mobile phase comprising Acetonitrile and Water (55:45 %v/v). The method employed a flow rate of 1.0 mL/min, UV detection at 262 nm, an injection volume of 10 µL, and a run time of 7 minutes. The drugs showed well-resolved, symmetrical peaks with retention times of approximately 2.3 minutes for Ofloxacin and 4.5 minutes for Satranidazole, with no interference from excipients. The method was validated according to ICH Q2(R1) guidelines, demonstrating excellent linearity ($R^2 > 0.999$), accuracy (recovery between 98–102%), precision (RSD < 2%), and specificity. The method showed adequate sensitivity with low limits of detection and quantitation, and robustness was confirmed under slight variations in chromatographic conditions.

Keywords: RP-HPLC, Ofloxacin and Satranidazole, Phenomenex Luna C18, simultaneous estimation, validation.

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

(a) it is not limited to volatile and thermally stable samples, and

(b) the choice of mobile and stationary phases is wider. [4] HPLC has numerous advantages like

- Simultaneous Analysis
- High Resolution
- High Sensitivity
- Good repeatability
- Small sample size
- Moderate analysis condition.
- Easy to fractionate the sample and purify. [5]

EXPERIMENTAL METHODS

INSTRUMENTS USED

1	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
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

S. No	Chemical	Brand names
1	Ofloxacin	Procured from Sun pharma, provided by Sura Pharma labs
2	Satranidazole	Procured from Sun pharma, 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 Ofloxacin and Satranidazole 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 2.25ml of the above Ofloxacin and 0.45ml of the Satranidazole 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.

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 450ml (45%) of Acetonitrile and 550ml of Water (55%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSIONS

(Optimized Chromatogram) (Standard)

Column	:	Phenomenex Luna C18 (4.6 x 150mm, 5µm)
Mobile phase	:	Acetonitrile and water (55:45 %v/v)
Flow rate	:	1ml/min
Wavelength	:	262 nm
Injection volume	:	10 µl
Run time	:	7 min

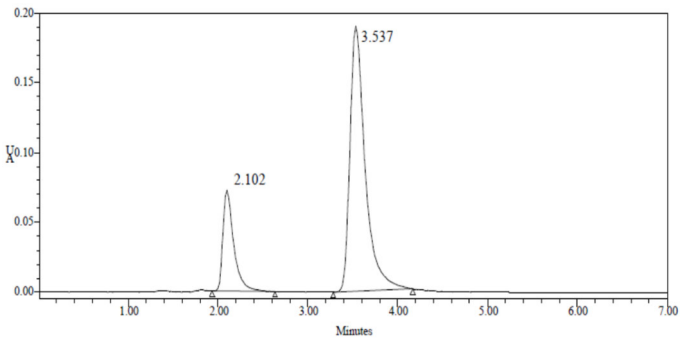

Fig 1: Optimized Chromatogram

Table 1: Peak results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	2.102	765789	69584		0.97	5587.0
2	Satranidazole	3.537	2532158	190049	2.97	1.26	5398.0

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

Optimized Chromatogram (Sample)

Column : Phenomenex Luna C18 (4.6 x 150mm, 5µm)
Mobile phase : Acetonitrile and water (45:55 %v/v)
Flow rate : 1ml/min
Wavelength : 250 nm
Injection volume : 10 µl
Run time : 7 min

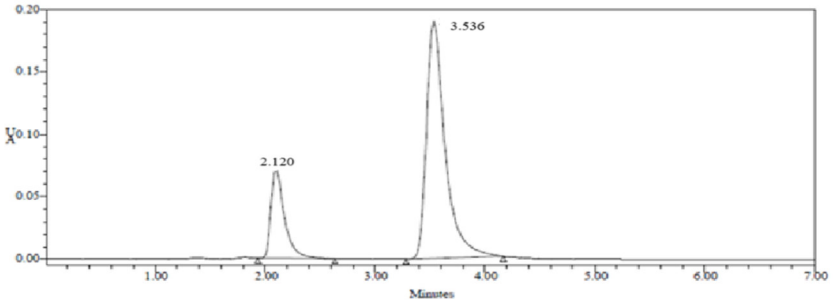

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	2.120	775684	13124		0.99	6365.0
2	Satranidazole	3.536	2658478	937405	5.06	1.23	7458.0

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

Blank

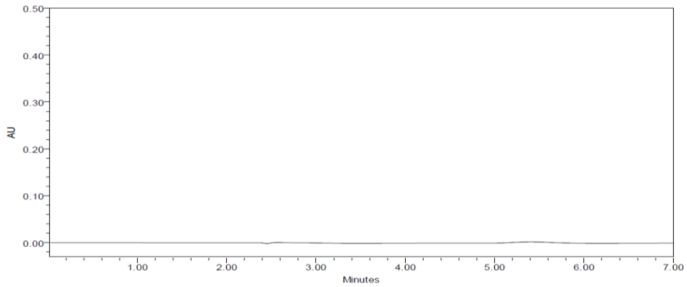

Fig 3: Chromatogram showing blank (mobile phase preparation)

System suitability

Table 3: Results of system suitability for Ofloxacin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ofloxacin	2.105	765843	69587	5589	1.9
2	Ofloxacin	2.103	766594	69854	5576	1.6
3	Ofloxacin	2.120	765487	70211	5658	1.6
4	Ofloxacin	2.110	765928	69213	5642	1.7
5	Ofloxacin	2.112	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			
% RSD			0.060932			

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 4: Results of system suitability for Satranidazole

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Satranidazole	3.537	2534658	190058	5365	1.2	2.07
2	Satranidazole	3.539	2536854	190052	5348	1.4	2.05
3	Satranidazole	3.536	2535879	190078	5389	1.5	2.0
4	Satranidazole	3.541	2533564	190035	5347	1.6	2.01
5	Satranidazole	3.542	2534214	190085	5364	1.6	2.01
Mean			2535034				
Std. Dev			1183.309				
% RSD			0.046678				

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.

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 Ofloxacin and Satranidazole in drug product.

Assay (Standard)

Table 5: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ofloxacin	2.105	759868	71255		1.7	5689	1
2	Satranidazole	3.537	2458754	215654	2.04	1.6	5362	1
3	Ofloxacin	2.103	759458	72541		1.7	5748	2
4	Satranidazole	3.539	2465885	226565	2.00	1.6	5452	2
5	Ofloxacin	2.120	759245	72584		1.7	5584	3
6	Satranidazole	3.536	2489578	221542	2.04	1.6	5456	3

Assay (Sample)

Table 6: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ofloxacin	2.120	756985	68958		0.98	7253	1
2	Satranidazole	3.536	2569856	198564	2.06	1.23	8836	1
3	Ofloxacin	2.110	758745	69857		1.05	6530	2
4	Satranidazole	3.541	2598654	195682	2.04	0.99	7270	2
5	Ofloxacin	2.112	756848	69588		1.7	7586	3
6	Satranidazole	3.542	2587454	192541	2.04	1.6	8371	3

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

The % purity of Ofloxacin and Satranidazole in pharmaceutical dosage form was found to be 99.8%.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:
Ofloxacin

Concentration µg/ml	Average Peak Area
0	0
15	205035
30	381239
45	561128
60	740162
75	909922

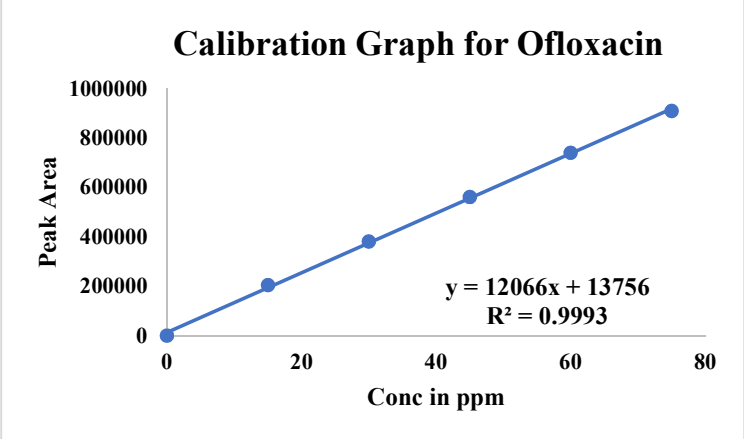

Fig 4: Calibration Graph for Ofloxacin

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

Y = mx + c
Slope (m) = 12066
Intercept (c) = 13756
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 13756. These values meet the validation criteria.

Satranidazole

Concentration µg/ml	Average Peak Area
0	0
10	757881
20	1457881
30	2132457
40	2901811
50	3501811

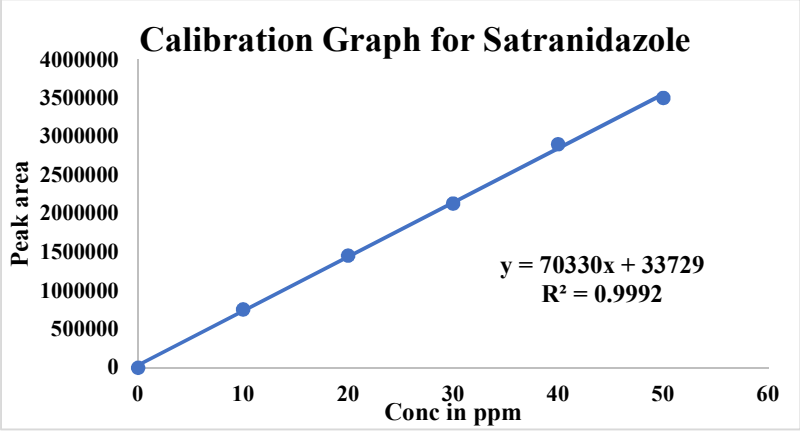

Fig 5: Calibration Graph for Satranidazole

LINEARITY PLOT

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

Y = mx + c
Slope (m) = 70330
Intercept (c) = 33729
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 33729. 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 7: Results of Repeatability for Ofloxacin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ofloxacin	2.112	766854	702564	5685	1.6
2	Ofloxacin	2.110	765884	698789	5584	1.4
3	Ofloxacin	2.120	765842	701235	5521	1.6
4	Ofloxacin	2.103	768985	700124	5525	1.9
5	Ofloxacin	2.105	765845	698986	5578	1.7
Mean			766682			

Std. Dev			1357.973			
% RSD			0.177123			

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 8: Results of method precision for Satranidazole

S. no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Satranidazole	3.542	2569865	2231111	5365	1.6
2	Satranidazole	3.541	2578474	2674210	5425	1.6
3	Satranidazole	3.536	2568985	2231261	5368	1.5
4	Satranidazole	3.539	2586845	2421301	5359	1.5
5	Satranidazole	3.537	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

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.

Intermediate precision
Day 1:

Table 9: Results of Intermediate precision for Ofloxacin

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ofloxacin	2.112	758955	68986	5785	1.6
2	Ofloxacin	2.110	759869	68957	5698	1.4
3	Ofloxacin	2.120	758985	68545	5689	1.6
4	Ofloxacin	2.103	756894	68952	5781	1.9
5	Ofloxacin	2.105	759854	68595	5785	1.7
6	Ofloxacin	2.102	756985	68952	5693	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

Acceptance criteria:

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

Table 10: Results of Intermediate precision for Satranidazole

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Satranidazole	3.542	2659852	190025	5485	1.5	2.04
2	Satranidazole	3.541	2648574	190048	5421	1.6	2.03
3	Satranidazole	3.536	2659865	190054	5468	1.6	2.01
4	Satranidazole	3.539	2658547	190078	5487	1.6	2.05
5	Satranidazole	3.537	2648981	190016	5492	1.6	2.02
6	Satranidazole	3.537	2654652	190057	5463	1.6	2.03
Mean			2655079				
Std. Dev			5242.086				
% RSD			0.197436				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table 11: Results of Intermediate precision Day 2 for Ofloxacin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ofloxacin	2.103	766895	69858	5586	1.5
2	Ofloxacin	2.105	765988	69854	5636	1.6
3	Ofloxacin	2.102	766532	69824	5432	1.6
4	Ofloxacin	2.112	766214	69875	5468	1.6
5	Ofloxacin	2.110	765897	69854	5546	1.9
6	Ofloxacin	2.120	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

Acceptance criteria:

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

Table 12: Results of Intermediate precision for Satranidazole

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Satranidazole	3.539	2653254	190110	5428	1.6	7.98
2	Satranidazole	3.537	2648985	190058	5452	1.6	6.4
3	Satranidazole	3.537	2658213	190142	5498	1.6	8.9
4	Satranidazole	3.542	2653652	190031	5442	1.5	8.3
5	Satranidazole	3.541	2648978	190058	5489	1.5	7.5
6	Satranidazole	3.536	2658985	190047	5463	1.6	5.3
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

Table 13: The accuracy results for Ofloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	392891.7	5	5.027	100.540%	100.354%
100%	781996	10	10.026	100.260%	
150%	1171988	15	15.038	100.253%	

Table 14: The accuracy results for Satranidazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	204962	15	15.156	101.040%	100.93%
100%	365018	30	30.378	101.260%	
150%	521064.3	45	45.218	100.484%	

Acceptance Criteria:

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

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.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Ofloxacin:

0.8µg/ml

Satranidazole:

0.9µg/ml

LIMIT OF QUANTITATION

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

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Ofloxacin:

1.5µg/ml

Satranidazole:

2.3µg/ml

Robustness

Variation in flow

Table 15: Results for Robustness

Ofloxacin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.102	5587	1.7
Less Flow rate of 0.9 mL/min	758698	2.330	5458	1.7
More Flow rate of 1.1 mL/min	7689584	1.950	5696	1.7
Less organic phase	758412	2.290	5586	1.4
More organic phase	769852	1.998	5355	1.5

Acceptance criteria:

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

Satranidazole

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	3.537	5398	1.6
Less Flow rate of 0.9 mL/min	2458692	3.885	5329	1.7
More Flow rate of 1.1 mL/min	2658642	3.263	5256	1.7
Less organic phase	2452148	4.435	5214	1.2
More organic phase	2653894	3.009	5524	1.0

Acceptance criteria:

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

SUMMARY AND CONCLUSION

A simple, precise, and accurate Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Ofloxacin and Satranidazole in bulk and pharmaceutical dosage forms. The chromatographic separation was carried out using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of Acetonitrile and Water in the ratio of 55:45 %v/v. The flow rate was maintained at 1.0 mL/min with detection at 262 nm using a UV detector. The injection volume was 10 µL, and the total run time was 7 minutes. Under the optimized conditions, both drugs were well separated with sharp, symmetrical peaks and satisfactory retention times approximately 2.3 minutes for Ofloxacin and 4.5 minutes for Satranidazole ensuring no interference from excipients or impurities.

The method was validated according to ICH Q2(R1) guidelines, demonstrating excellent linearity ($R^2 > 0.999$) over the tested concentration range. Accuracy was confirmed through recovery studies, which showed values within 98–102%, while precision studies revealed relative standard deviations (RSD) of less than 2%, indicating good repeatability and reproducibility. The method was also found to be specific, as no interference from formulation components was observed, and sensitive, with appropriately low LOD and LOQ values. Furthermore, robustness testing confirmed that minor changes in chromatographic conditions did not significantly affect the results.

In conclusion, the proposed RP-HPLC method is validated, reliable, and suitable for routine quality control analysis of Ofloxacin and Satranidazole in both bulk drugs and combined dosage forms. The short analysis time, high accuracy, and clear resolution make it highly effective for simultaneous estimation in pharmaceutical settings.

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