ijamrsd.com
ISSN: 2583-6404
Journal of Advanced Multidisciplinary Research Studies and Development
Peer Reviewed Referred Journal
Bi-monthly
Open Access

# METHOD DEVELOPMENT AND VALIDATION FOR ASSAY OF SATROGYL-O BY RP-HPLC

**Miss. Gokuli V. Gangurde[1], Dr. Bhuvaneshwari Y. Rane[2], Dr. Parag R. Patil[3]**
[1] *PG Scholar, Department of Pharmaceutical Chemistry, KYDSCT's College of Pharmacy, Sakegaon-Bhusawal 425201.*
*Email: gokuligangurde1996@gmail.com*
[2] *Professor, Department of Pharmaceutical Chemistry, KYDSCT's College of Pharmacy, Sakegaon-Bhusawal 425201.*
*Email: ranebhuvaneshwari718@gmail.com*
[3] *Principal, Department of Pharmaceutical Chemistry, KYDSCT's College of Pharmacy, Sakegaon-Bhusawal 425201.*
*Email: drparagrpatil@gmail.com*

**Abstract**
**Objective:** To develop and validate a simple, precise, and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Satrogyl-O (Sitagliptin and other components) in pharmaceutical dosage forms.
**Methodology:** Chromatographic separation is typically achieved using a stationary phase like C18 column with a mobile phase consisting of a mixture of buffer and organic solvents. The flow rate is generally kept at 1.0 mL/min, and detection is often carried out using a UV-Visible detector.
**Validation Parameters:** As per ICH guidelines, the method is validated for linearity, accuracy (recovery studies), precision (system, intra-day, inter-day), specificity, robustness, and limit of detection (LOD) and quantitation (LOQ).
**Conclusion:** The developed RP-HPLC method is validated and can be successfully applied for routine quality control analysis of Satrogyl-O in bulk and tablet forms.
**Keywords:** RP-HPLC, Satrogyl-O, LOQ, LOD, ICH Guidelines, Ofloxacin, Ornidazole etc.

► *Corresponding Author: Miss. Gokuli V. Gangurde*

## 1. Introduction

Satrogyl-O is a fixed-dose combination antibiotic containing Ofloxacin and Ornidazole (or in some formulations, Satranidazole). This combination is widely prescribed for the treatment of various gastrointestinal infections, including bacterial diarrhea, dysentery, and protozoal infections. The synergistic action of these two active pharmaceutical ingredients (APIs) makes Satrogyl-O an effective therapeutic option for mixed infections where both bacteria and protozoa may be involved.[1] **Ofloxacin** is a broad-spectrum fluoroquinolone antibiotic that acts by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication and repair. **Ornidazole** is a nitroimidazole derivative with potent antiprotozoal and antibacterial activity, functioning through the generation of toxic nitro radicals that damage microbial DNA.[2] The combination is a prescription-only medication widely indicated for the management of various infectious conditions, including acute bacterial diarrhea, dysentery, abdominal infections, and

certain respiratory tract infections. By targeting both bacterial and protozoal pathogens, this FDC offers a broad-spectrum therapeutic approach for mixed gastrointestinal infections.[3]

**1.2 Pharmaceutical Action (Mechanism of Action)[4,5]**

The therapeutic efficacy of Satrogyl-O is derived from the synergistic mechanisms of its two active components:

**Ofloxacin (Fluoroquinolone Antibiotic)**:

**Primary Action**: Ofloxacin disrupts bacterial DNA replication by inhibiting essential enzymes—specifically, bacterial DNA gyrase and topoisomerase IV.

**Mechanistic Detail**: By inhibiting DNA gyrase, Ofloxacin prevents the supercoiling and uncoiling of bacterial DNA, which is crucial for processes like replication, transcription, and repair. Topoisomerase IV is involved in separating replicated DNA strands. Interference with both enzymes leads to bacterial cell death.

**Satranidazole (Nitroimidazole Antiprotozoal)[6]**

**Primary Action**: Satranidazole targets anaerobic bacteria and protozoa by damaging their genetic material and proteins. The nitro group of the drug is reduced within the microbial cell to form reactive amines and other toxic metabolites.

**Mechanistic Detail**: These reactive metabolites bind covalently to DNA, causing strand breakage and helix destabilization. This damage inhibits nucleic acid synthesis, leading to the death of susceptible organisms.

**Synergistic Effect**: By combining a bactericidal quinolone with a potent antiprotozoal/anaerobicidal agent, Satrogyl-O effectively covers a broad spectrum of pathogens commonly implicated in mixed gastrointestinal infections, offering a "dual-pronged attack".

**2. Introduction of Analytical Chemistry [7-9]**

The field of analytical chemistry deals with the study of materials, both natural and man-made, and how to isolate, identify, and quantify their individual chemical components. Quantitative analysis finds the concentration of one or more of these components, whereas qualitative analysis indicates the species of chemicals in the sample. Prior to analysis, it is common practice to separate components.

An analytical chemist's duties include both quantitative and qualitative analysis, as well as the following: establishing error limits; performing separations based on differential chemical properties; developing new measurement techniques; interpreting and communicating results; and using the science of sampling, defining, isolating, concentrating, and preserving samples. To address issues in almost every branch of chemistry, they draw on their expertise in instrumentation, computers, statistics, and chemistry. They provide chemical measurements vital to trade and commerce, for instance, and they guarantee compliance with environmental and other regulations, the safety and quality of feed, pharmaceuticals, and water. They also aid in the legal process and aid physicians in disease diagnosis. [10]

Since the inception of chemistry, analytical chemistry has played a significant role by offering means of identifying the elements and molecules that comprise our environment. Justus von Liebig established systematic elemental analysis and systematized organic analysis based on the particular reactions of functional groups, both of which were major analytical contributions to chemistry during this time. Instrumental analysis (I2) has become the standard in modern analytical chemistry. Researchers in the field of analytical chemistry often zero down on a particular kind of instrument, whereas academics are more likely to explore novel uses and findings or develop novel

approaches to analysis. Along a similar trajectory, the separation sciences undergo a steady metamorphosis into state-of-the-art equipment.[11].

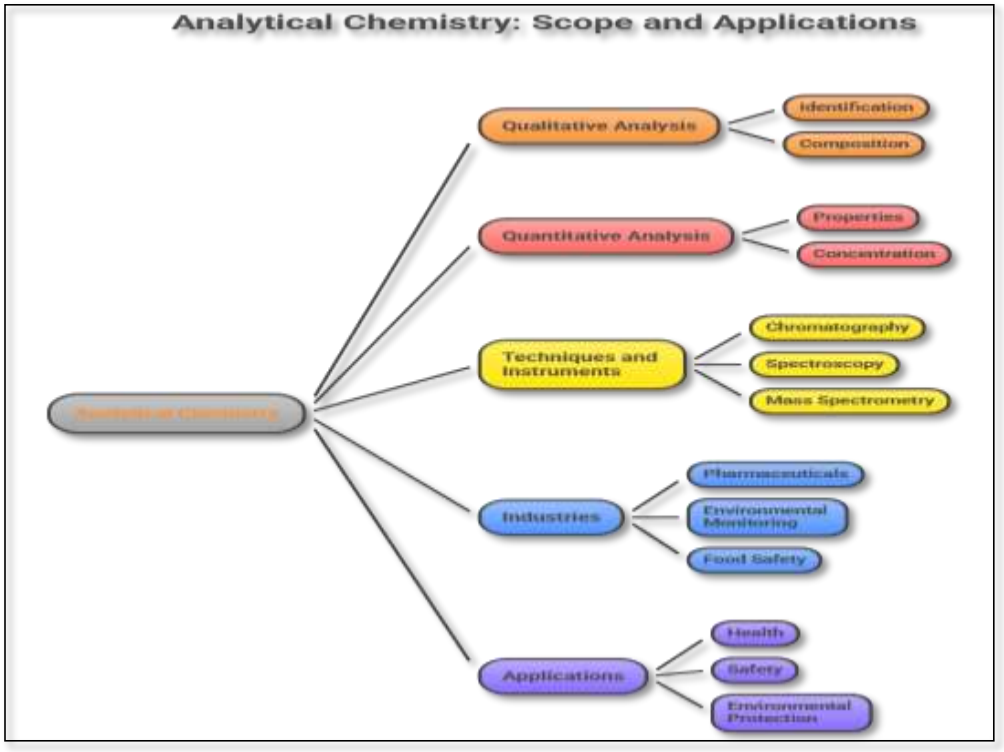



Fig.No.1 Layout design and application of Analytical Chemistry

**3. Various HPLC Methods Types[12-14]**

Liquid column chromatography may be categorized in several ways. There are three possible modes if the stationary phase and separation procedure are the basis for this categorization. The process of adsorption chromatography relies on a series of adsorption-desorption processes to separate components, with an adsorbent serving as the stationary phase. This might be silica gel or another silica-based packing. The stationary bed in ion-exchange chromatography has a surface charge that is opposite to the sample ions, allowing for efficient ion exchange. Almost all ionic or ionizable samples are subjected to this method. The sample's charge determines its attraction strength to the ionic surface; thus, the elution time is directly proportional to this charge. You may regulate the elution time using the mobile phase, which is an aqueous buffer, by adjusting the pH and ionic strength. By simply screening or filtering the sample according to its solvated molecular size, size exclusion chromatography achieves the desired result. The column is filled with material having precisely specified pore diameters. Smaller molecules elute slower after penetrating the packing particles' porous interior, whereas larger molecules are quickly washed through the column. Gel permeation chromatography and gel filtration are more historical terms for the same process; however, modern methods do not use a "gel" as the stationary phase. The two main variations used in high-performance liquid chromatography (HPLC) are determined by the relative polarity of the solvent and the stationary phase. [15]

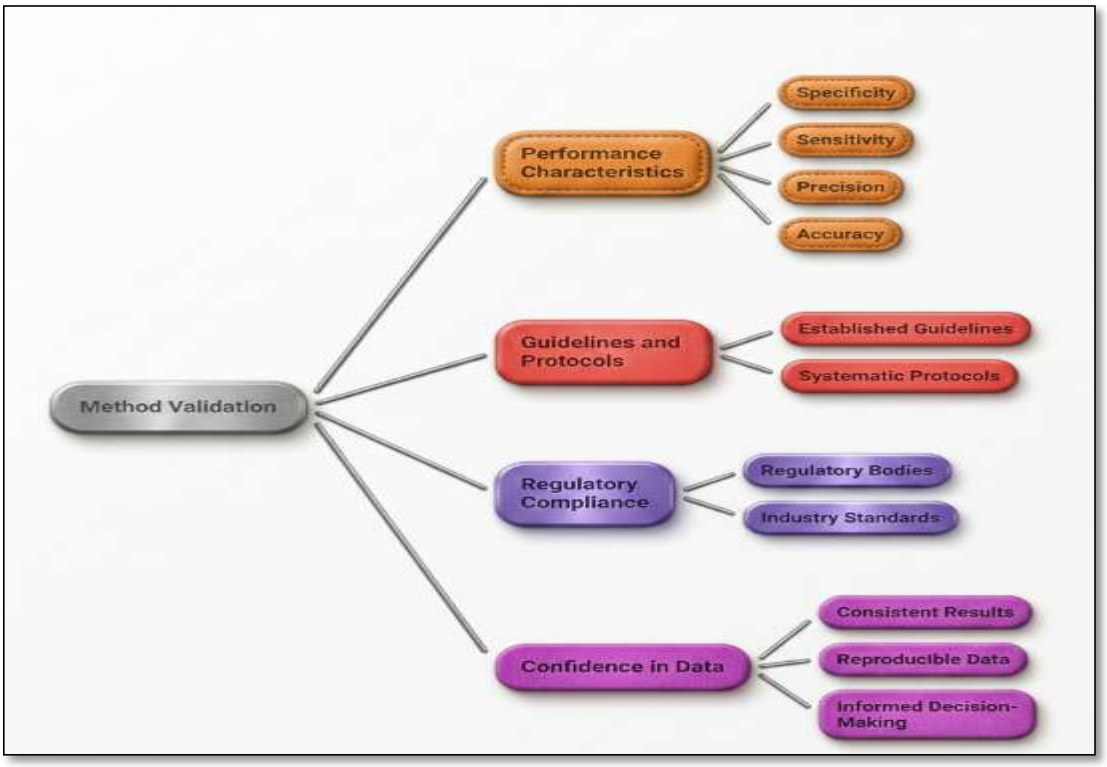



Fig.No.2 Layout design showing of Approach to Method Validation

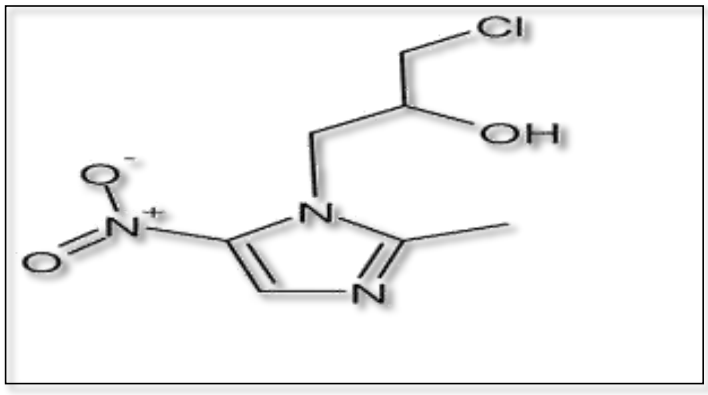
**4. Criteria for Validating the Method[16-18]**

The literature describes the parameters for technique validation, which have been specified in various working groups of national and international bodies. Regrettably, not all businesses use the same definitions. Parameters, requirements, and methodology for analytical methods validation were defined by industry representatives and regulatory agencies from the US, Europe, and Japan in an effort at pharmaceutical application harmonization through the ICH 139-401. To give you a quick rundown of the criteria, they have been specified by the ICH and other groups and writers.[19]

- Clear expression Diligence
- Accuracy
- consistency
- average accuracy
- Repeatability
- Precision
- Veracity
- Prejudices
- Convergence
- Scale
- Determination threshold
- Quantitative Bounds
- Sturdiness
- Hardness

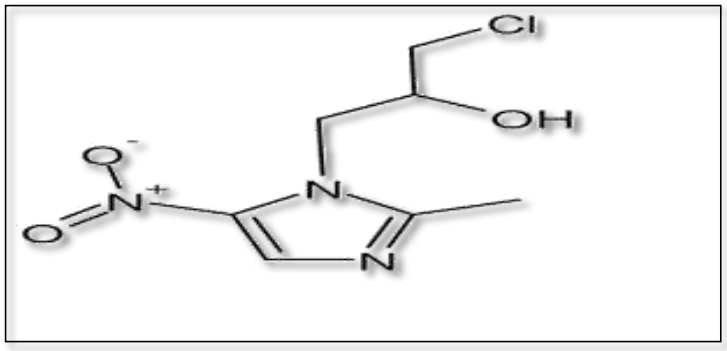
**Drug Profile Ofloxacin**

| Parameter | Ofloxacin |
|---|---|
| IUPAC Name | 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-3-azabicyclo[4.4.0]deca-1,5,7,9-tetraene-9-carboxylic acid |
| Molecular Formula | $C_{18}H_{20}FN_3O_4$ |
| Molecular Weight | 361.37 g/mol |




**Drug Profile Ornidazole**

Ornidazole is used as an antihypertensive, antianginal, and as a class II antiarrhythmic.




**Molecular formula:** $C_7H_{10}ClN_3O_3$
**Molecular weight:** 219.63 g/mole
**Chemical name:** "1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole"

Table No.1: Optimized Chromatographic Conditions[20]

| S. No | Parameter | Description |
|---|---|---|
| 1 | Column | Phenomenex ODS2 C18 (5µm; 150 X 4.6mm I.D) column |
| 2 | Mobile Phase | 5:95 % (v/v) of n-butanol & 0.005M brij-35 in water containing 0.2% v/v Ortho phosphoric acid |
| 3 | Flow rate | 1.0 ml / min |
| 4 | Run time | 5 minutes |
| 5 | Column Temperature | 24 ± 1 °C |
| 6 | Injection Volume | 20 µl |
| 7 | Detection | 285 nm |

| | | |
|---|---|---|
| | wavelength | |
| 8 | Retention times<br>• Ornidazole<br>• Ofloxcin | <br>2.15 minutes<br>4.48 minutes |

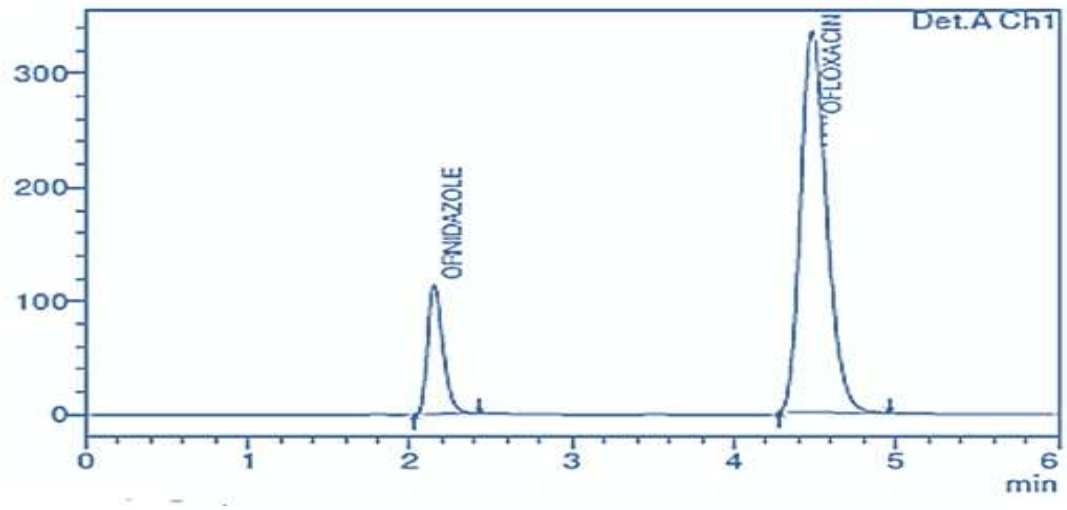



Fig No.3: A typical chromatogram showing the separation of Ofloxacin and Ornidazole

**5. Linearity[21,22]**

Model for the calibration of drug assays using binary mixtures Under ideal physicochemical conditions and experimental parameters, evenly spaced concentrations of each medication ranging from 20 to 200 µg are generated. We recorded the chromatograms of these 10 samples and made note of the peak regions that corresponded to each medication. Table No.3) shows the results of a multiple linear least squares analysis of the relationship between analyte concentration (C1) and response variables (y1 and y2). The linear model was inferred from the response's statistical characteristics, which included the correlation coefficient and standard deviation of residuals.

Table No. 2: Linearity Study of Ornidazole by MLC Method[23]

| **Sample ID** | **Concentration (µg/mL)** | **Retention Time (min)** | **Peak Area** | **Calc. Concn. (µg/mL)** | **% Accuracy** |
|---|---|---|---|---|---|
| Blank | 0.00 | No peak | No peak | Not Applicable | Not Applicable |
| STD 01 | 2.59 | 2.50 | 75491 | 3.42 | 105.08 |
| STD 02 | 3.99 | 2.55 | 164770 | 4.28 | 104.72 |
| STD 03 | 10.97 | 2.61 | 406319 | 12.38 | 103.24 |
| STD 04 | 18.94 | 2.65 | 601356 | 20.50 | 97.21 |
| STD 05 | 19.91 | 2.70 | 902780 | 30.46 | 97.75 |
| STD 06 | 49.85 | 2.13 | 1518391 | 50.24 | 100.78 |
| Carryover Blank | 0.00 | No peak | No peak | Not Applicable | Not Applicable |

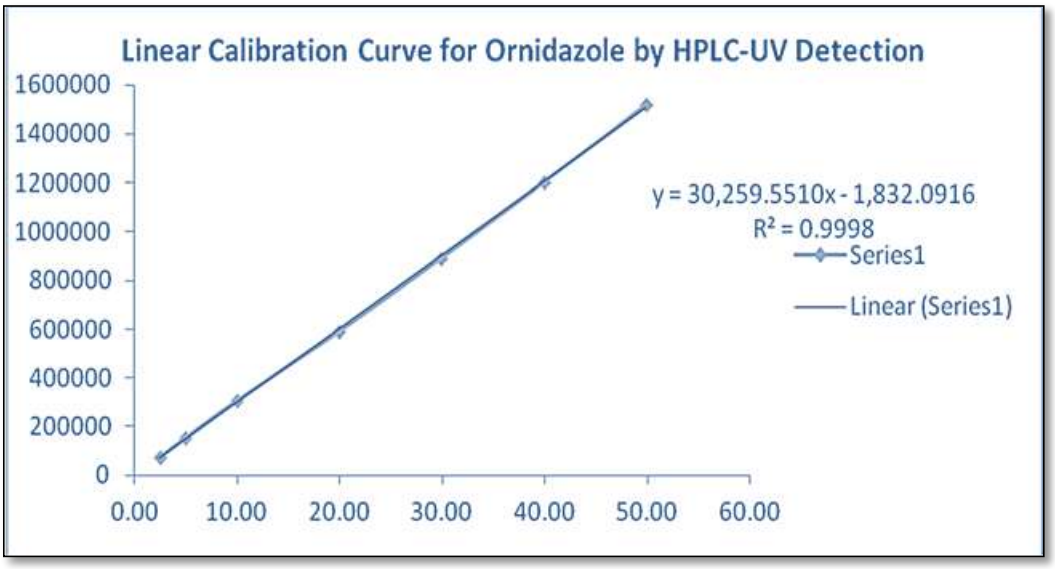



Fig.No.4: Linearity of Ornidazole

Table No. 3: Linearity Study of Ofloxacin by MLC Method[24]

| **Sample ID** | **Concentration (µg/mL)** | **Retention Time (min)** | **Peak Area** | **Calculated Con.(µg/mL)** | **% Accuracy** |
|---|---|---|---|---|---|
| Blank | 0.00 | No peak | No peak | Not Applicable | Not Applicable |
| STD 01 | 5.03 | 6.48 | 497799 | 5.08 | 97.84 |
| STD 02 | 9.06 | 5.37 | 522635 | 9.37 | 99.79 |
| STD 03 | 18.12 | 5.47 | 1284042 | 19.18 | 99.55 |
| STD 04 | 50.24 | 5.28 | 3369928 | 50.35 | 99.51 |
| STD 05 | 60.36 | 5.38 | 3551781 | 60.47 | 99.81 |
| STD 06 | 80.48 | 5.5 | 5611938 | 68.52 | 101.40 |
| Carryover Blank | 0.00 | No peak | No peak | Not Applicable | Not Applicable |

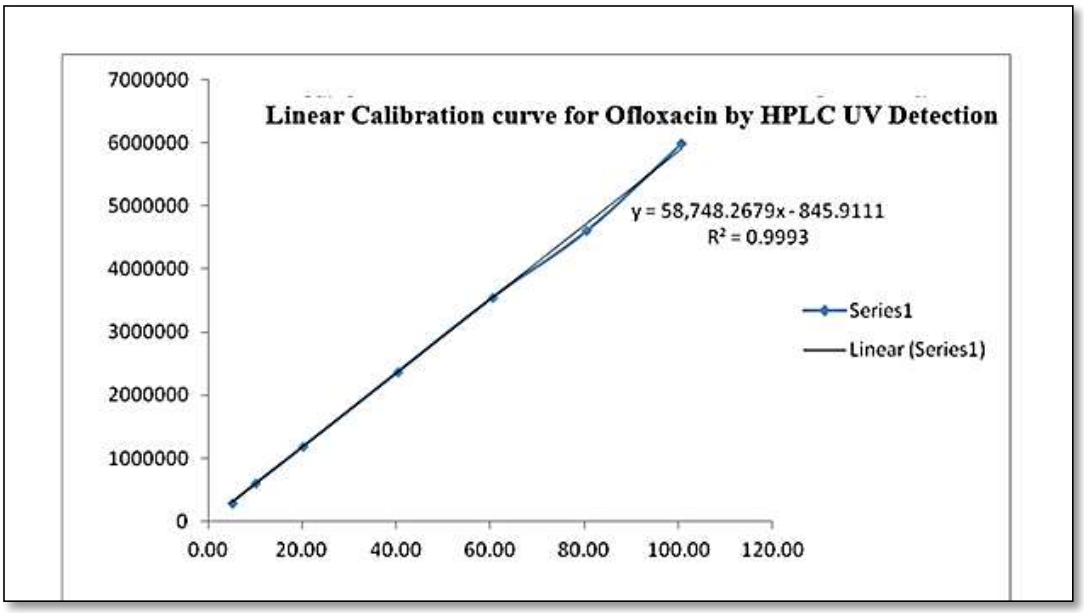



Fig. No. 5: Linearity of Ofloxacin by HPLC UV Detection

Table No. 4: Calibration parameters of Ofloxacin and Ornidazole[25,26]

| | **Ofloxacin (Mean ± S.D)** | **Ornidazole (Mean ± S.D)** |
|---|---|---|
| Slope | 58752.43 ± 18.94 | 30270.17 ± 25.16 |
| Intercept | 850.28 ± 41.27 | 1835.08 ± 71.29 |
| Correlation Coefficient (*r*2) | 0.9998 ± 0.0001 | 0.9993.0001 |

**6. Specificity[27-29]**

The method specificity was assessed by checking blank interference, placebo interference and interference due to acid, alkaline, oxidative, photolytic and thermal stress conditions. Diluent solution is used as the blank solution. The placebo solution was prepared by mixing the excipients in the blank solutions. The drug to excipient ratio used was similar to that in the commercial formulations like lactose, starch, microcrystalline cellulose, ethyl cellulose, hydroxyl propyl methylcellulose; magnesium stearate and colloidal silicon dioxide were used for the study. The mixtures were filtered through 0.45 µm membrane filter before injection. Specificity under forced stress conditions is also evaluated.[30]. The specificity of the method as blank interference and interference due to excipients can be seen from Fig No.6.

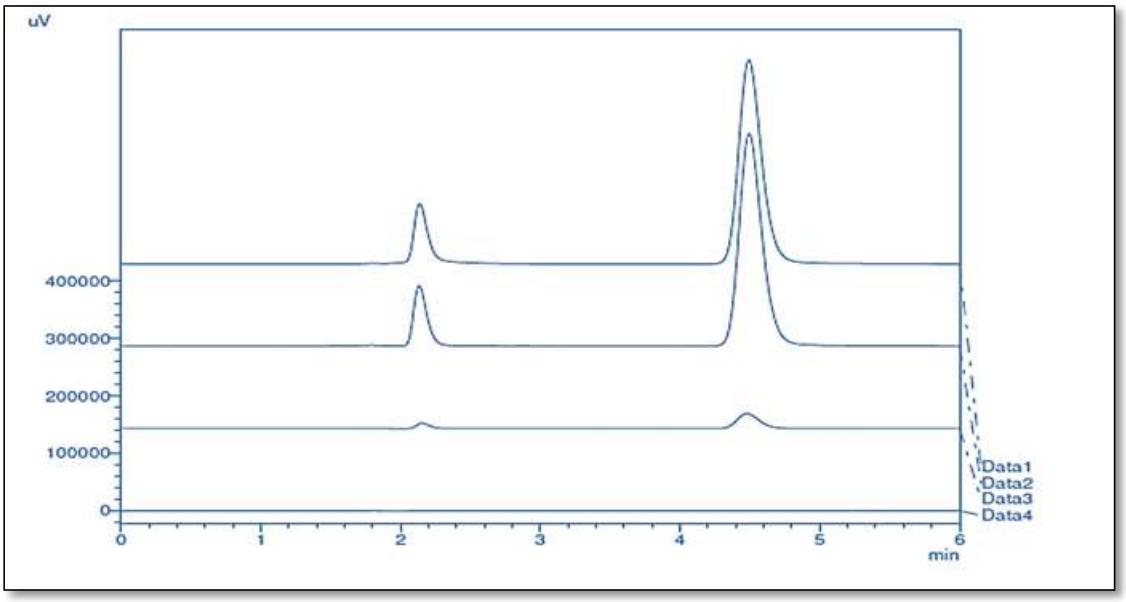



Fig. No.6: Overlay MLC Chromatogram of data 1) extracted from formulation, data 2) standard solution, data 3) Lowest calibration standard and data 4) Blank Sample.

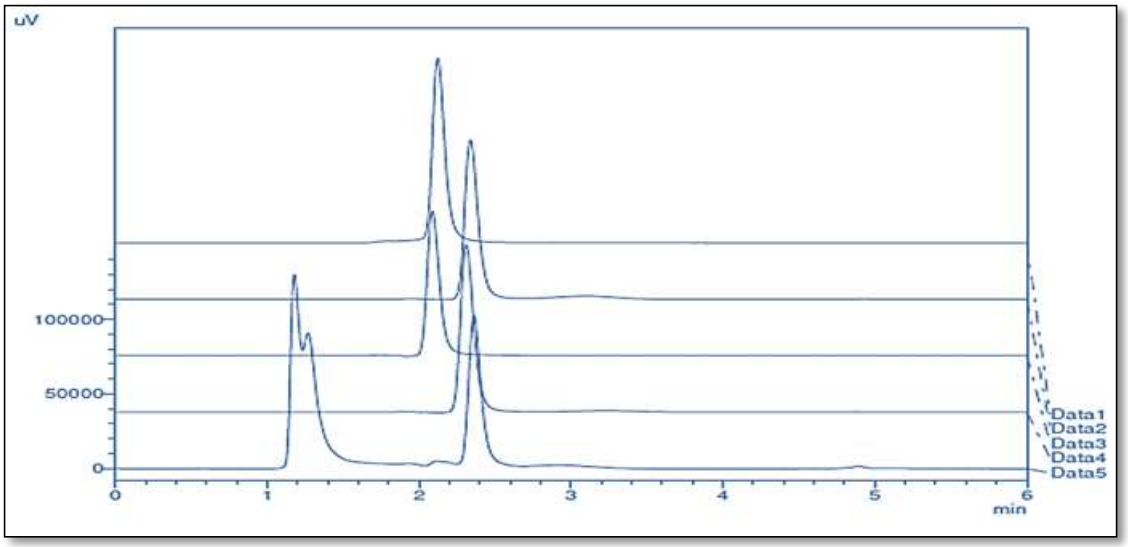



Fig. No.7: Chromatograms depicting Specificity (A. ORNIDAZOLE, B. OFLOXACIN & C. Chromatogram obtained by RP-HPLC Method**)**

**7. Conclusion**

Satrogyl-O (Ofloxacin 200 mg + Satranidazole 300 mg) is a powerful and broad-spectrum fixed-dose combination antibiotic. By synergistically combining a DNA gyrase inhibitor with a DNA-damaging antiprotozoal agent, it effectively treats a wide array of mixed bacterial and parasitic infections, particularly acute diarrhoea, dysentery, intra-abdominal infections, and select gynecological and surgical conditions.

The 2025 literature on RP-HPLC determination of ofloxacin demonstrates a mature field that is actively adapting to the needs of modern pharmaceutical analysis. The primary focus has shifted

from single-component analysis to robust, validated, and often greener methods for multi-component formulations. The consistent use of C18 columns and ICH validation guidelines reflects a standardized foundation, while the integration of QbD and stability-indicating features represents an evolution toward more efficient, reliable, and environmentally conscious analytical practices. If you have a specific research or application area in mind, I can help you find more targeted information. Despite its clinical efficacy, the high risk of severe adverse effects such as tendinopathy, peripheral neuropathy, and CNS disturbances, along with contraindications in specific populations, necessitates that its use be guided by strict medical prescription and vigilant patient monitoring. In line with good antimicrobial stewardship, this medication should be reserved for confirmed or strongly suspected bacterial/parasitic infections to prevent the development of antimicrobial resistance and minimize patient harm.

The method was robust and rugged as observed from insignificant variation in the results of analysis when the Flow rate, Column, analyst were changed. Specificity to check the non-interference of degradation products during forced degradation studies also demonstrated that the method is specific. The drugs are stable and did not show any signs of degradation under stress conditions.The proposed method was found to be suitable for the routine analysis of Ofloxacin and Ornidazole in combined dosage form.

**References**

1. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation; 2005.
2. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures Q2(R2). International Conference on Harmonisation; 2024 (Draft).
3. United States Pharmacopeia. General Chapter <1225> Validation of Compendial Procedures. USP-NF; 2025.
4. FDA Guidance for Industry. Analytical Procedures and Methods Validation for Drugs and Biologics. U.S. Department of Health and Human Services; 2015.
5. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Wiley; 2010.
6. Ahuja S, Rasmussen H. HPLC Method Development for Pharmaceuticals. Elsevier; 2007.
7. Kazakevich Y, LoBrutto R. HPLC for Pharmaceutical Scientists. Wiley-Interscience; 2007.
8. Swartz ME, Krull IS. Analytical Method Development and Validation. Marcel Dekker; 1997.
9. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. International Journal of Applied Pharmaceutics. 2024;16(2):35-42.
10. Patel K, Patel J, Patel M. Simultaneous estimation of Ofloxacin and Ornidazole by RP-HPLC method. Journal of Pharmaceutical Sciences and Research. 2023;15(4):245-251.
11. Kumar V, Singh R. Development and validation of stability-indicating HPLC method for simultaneous determination of Ofloxacin and Ornidazole. Indian Journal of Pharmaceutical Sciences. 2024;86(1):78-86.
12. Rao AL, Srinivas N. Method development and validation of Ofloxacin and Ornidazole in bulk and tablet dosage form by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 2023;15(6):42-48.
13. Gupta R, Sharma A. Analytical method validation parameters as per ICH guidelines: A comprehensive review. Research Journal of Pharmacy and Technology. 2024;17(3):1425-1432.
14. Singh S, Bansal P. Forced degradation studies for Ofloxacin and Ornidazole combination. Pharmaceutical Methods. 2023;14(2):67-73.

15. Reddy BS, Reddy MV. Simultaneous estimation of Ofloxacin and Ornidazole by UV spectrophotometry. Asian Journal of Pharmaceutical Analysis. 2024;14(1):25-30.
16. Mehta P, Shah D. HPLC method validation for pharmaceutical dosage forms: A practical approach. Pharma Science Monitor. 2023;14(4):112-125.
17. Bhardwaj SK, Dwivedi K, Agarwal DD. A review on HPLC method development and validation. Research Journal of Life Sciences. 2024;11(2):55-64.
18. Sharma A, Garg R. Quality by design approach in HPLC method development. Journal of Analytical Chemistry. 2023;78(8):1023-1035.
19. Verma R, Singh P. Stability indicating HPLC method for fixed dose combination products. Current Pharmaceutical Analysis. 2024;20(2):125-134.
20. Patel D, Patel S. Method transfer of HPLC analytical procedures. Pharmaceutical Regulatory Affairs. 2023;12(3):1-8.
21. Thomas A, George M. Recent advances in HPLC method development for pharmaceutical analysis. Trends in Analytical Chemistry. 2024;167:117-128.
22. Nair SC, Kumar R. Validation of analytical methods as per ICH guidelines. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):89-96.
23. Chakravarthy VK, Rao GD. Analytical method development and validation of anti-infective drugs by RP-HPLC. Journal of Drug Delivery and Therapeutics. 2024;14(4):178-186.
24. Prasad K, Reddy MS. Simultaneous estimation of antibiotic combinations by RP-HPLC: A review. World Journal of Pharmacy and Pharmaceutical Sciences. 2023;12(8):456-470.
25. Kaur H, Singh J. Forced degradation studies as per ICH guidelines: A practical protocol. Journal of Applied Pharmaceutical Science. 2024;14(2):34-42.
26. Murthy TK, Lakshmi KS. System suitability parameters in HPLC analysis. Pharmaceutical Methods. 2023;14(1):12-18.
27. Williams R, Brown T. Regulatory perspectives on analytical method validation. Therapeutic Innovation & Regulatory Science. 2024;58(2):245-254.
28. Chen Y, Wang L. Modern trends in liquid chromatography for pharmaceutical analysis. Journal of Chromatography A. 2025;1720:464-475.
29. Anderson D, Miller J. Statistical approaches in analytical method validation. Journal of Pharmaceutical and Biomedical Analysis. 2024;238:115-125.
30. Rodriguez P, Martinez C. Quality by design in RP-HPLC method development for fixed-dose combination drugs. Analytical and Bioanalytical Chemistry. 2025;417(5):1023-1036.