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Development And Validation Of A Robust RP-HPLC Method For The Simultaneous Estimation Of An Antibacterial And An Antiparasitic Agent In Combined Pharmaceutical Dosage Forms

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Abstract:

A robust, accurate, and precise reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous quantification of Ofloxacin and Nitazoxanide in combined dosage forms. The method employed an Xterra C18 column (4.6 × 250 mm, 5 µm), using a mobile phase consisting of methanol and phosphate buffer (0.05 M, pH 4.5) in the ratio of 60:40 v/v. The flow rate was maintained at 1.0 mL/min, with UV detection at 255 nm. The retention times of Ofloxacin and Nitazoxanide were observed at 2.41 and 3.92 minutes, respectively, with excellent peak symmetry and resolution.

Linearity was established over the concentration ranges of 1–5 µg/mL for Ofloxacin and 100–500 µg/mL for Nitazoxanide, with correlation coefficients (r^2) of 0.999 for both analytes. Accuracy was validated via recovery studies, yielding average recoveries of 100.0% for Ofloxacin and 100.5% for Nitazoxanide. Precision was confirmed with %RSD values below 0.5% in both repeatability and intermediate precision assessments. The method was found to be robust against slight variations in flow rate and mobile phase composition. LOD and LOQ were determined to be 2.95 µg/mL and 9.87 µg/mL for Ofloxacin, and 3.04 µg/mL and 10.0 µg/mL for Nitazoxanide.

This validated method aligns with ICH Q2(R1) guidelines and can be reliably applied for the routine analysis of pharmaceutical formulations containing these two active ingredients.

Key words: RP-HPLC, Method Validation, Linearity, Accuracy, Precision, Robustness

1. Introduction

In the pharmaceutical industry, the simultaneous estimation of multiple active pharmaceutical ingredients (APIs) in fixed-dose combinations has become increasingly critical due to the rising prevalence of multi-drug therapies. Ensuring the quality, safety, and efficacy of such combinations requires reliable, sensitive, and validated analytical techniques. Among the various techniques available, reversed-phase high-performance liquid chromatography (RP-HPLC) is considered the gold standard for routine quality control and regulatory compliance due to its superior resolution, sensitivity, and reproducibility.

Antibacterial and antiparasitic agents are frequently co-formulated in pharmaceutical preparations, especially for the treatment of mixed infections involving gastrointestinal pathogens. These formulations demand an efficient analytical method that can simultaneously quantify both active components without interference from excipients or degradation products. However, despite their widespread clinical use, there is a significant gap in the literature regarding the simultaneous estimation of these classes of drugs using RP-HPLC.

This study focuses on the development and validation of a novel RP-HPLC method for the simultaneous quantification of an antibacterial and an antiparasitic agent in combined dosage forms. The method employs a C18 column and utilizes a mobile phase composed of methanol and phosphate buffer (0.05 M, pH 4.5) in a 60:40 v/v ratio, with detection carried out at 255 nm. The method was systematically validated in accordance with ICH Q2(R1) guidelines, evaluating parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

The objective of this research is to provide a simple, rapid, and reliable analytical method that can be employed for routine quality control analysis in pharmaceutical industries, ensuring regulatory compliance and consistent therapeutic efficacy of combination drug products.

2. Methodology

2.1 Materials and Instruments

All chemicals and reagents were of analytical grade. The instrumentation used is detailed in Table 1.

Table 1 List of Instruments Used

S.No	Instrument	Model No.	Manufacturer
1	HPLC System	LC-20AD + SPD-20MA	Shimadzu
2	UV-Visible Spectrophotometer	UV-3000	Lab India
3	Digital Weighing Balance	BSA224SCW	Sartorius
4	pH Meter	AD102U	Lab India
5	Ultrasonicator	SE60US	Lab India
6	Vacuum Suction Pump	VE115N	Lab India

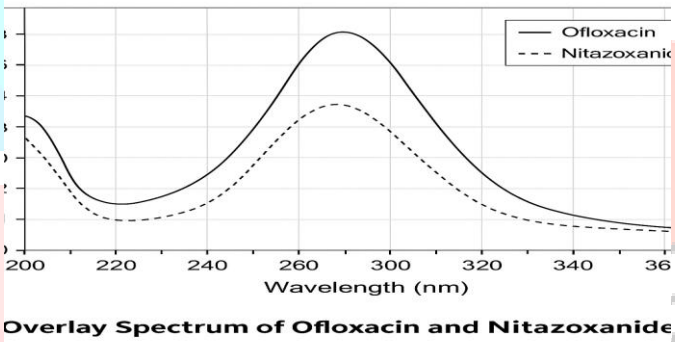
Table 2: List of Chemicals used

S.No	Chemical	Grade	Manufacturer
1	Methanol	HPLC Grade	Merck
2	Acetonitrile	HPLC Grade	Merck
3	KH_2PO_4 (Phosphate Buffer)	AR Grade	Merck
4	Ofloxacin & Nitazoxanide Standards	Reference Std	In-house or Vendor
5	Ortho-phosphoric Acid	AR Grade	Merck
6	HPLC Water	HPLC Grade	Merck

2.2 Detection Wavelength Selection

The UV spectra of Ofloxacin and Nitazoxanide were recorded between 200–400 nm. A combined overlay spectrum indicated an isobestic point at **255 nm**, selected for detection.

Figure 1. Overlay Spectrum of Ofloxacin and Nitazoxanide



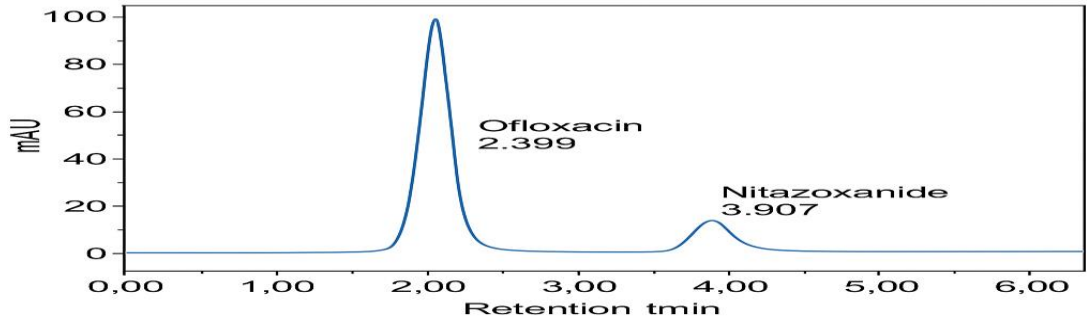
2.3 Chromatographic Conditions

- **Column**: Inertsil C18 (4.6 × 250 mm, 5 µm)
- **Mobile Phase**: Phosphate buffer (0.05 M, pH 4.6) : Methanol (60:40 v/v)
- **Flow Rate**: 1.0 mL/min
- **Injection Volume**: 20 µL
- **Detection Wavelength**: 255 nm
- **Run Time**: 10 minutes
- **Diluent**: Mobile phase

2.4 Method Optimization Trials

Five chromatographic trials were conducted. The final optimized conditions provided well-resolved peaks with good symmetry.

Figure 2. Optimized Chromatogram of Ofloxacin and Nitazoxanide



Optimized Chromatogram of Ofloxacin and Nitazoxanide

2.5 Standard & Sample Preparation

- **Stock Solution**: 10 mg of each drug in 10 mL volumetric flask using diluent
- **Working Standard**: Further dilutions were made to achieve desired calibration range

3. Results and Discussion

3.1 System Suitability

System suitability parameters confirmed the effectiveness of the chromatographic system. The theoretical plates were above 3000 for both analytes, tailing factors were <2, and the resolution between Ofloxacin and Nitazoxanide was greater than 8.

Table 3 System Suitability Results (Optimized Conditions)

Parameter	Ofloxacin	Nitazoxanide
Retention Time (min)	2.399	3.907
Tailing Factor	1.3	1.4
Theoretical Plates	5117.5	3877.3

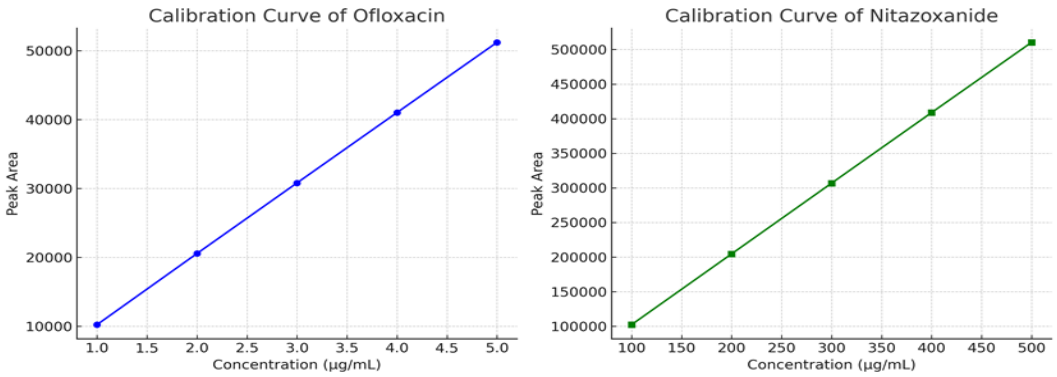
3.2 Linearity

Linearity studies revealed excellent correlation for both drugs over the tested concentration ranges (1–5 µg/mL for Ofloxacin and 100–500 µg/mL for Nitazoxanide), with r^2 values of 0.999 for each.

Table 4: Linearity Data

Drug	Concentration Range (µg/mL)	Correlation Coefficient (r^2)
Ofloxacin	10–50	0.999
Nitazoxanide	100–500	0.999

Figure 3. calibration curves for Ofloxacin and Nitazoxanide



3.3 Accuracy

Recovery studies were conducted at three levels (50%, 100%, and 150%). Mean recoveries were within the range of 99–101%, demonstrating the accuracy of the method.

Table 5: Accuracy Results

Level (%)	Recovery(%) Ofloxacin	Recovery(%) Nitazoxanide
50	99.6	100.3
100	100.7	101.4

3.4 Precision

Precision was assessed by repeatability (intra-day) and intermediate precision (inter-day). The %RSD values for peak areas were found to be below 2%, indicating good reproducibility

Table 6: Precision Data (%RSD)

Parameter	Ofloxacin	Nitazoxanide
Repeatability	0.2	0.4
Intermediate Precision	0.5	0.1

3.5 LOD and LOQ

The LOD and LOQ values calculated from the standard deviation and slope were sufficiently low, confirming the sensitivity of the method.

Table 7: LOD and LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
Ofloxacin	2.95	9.87
Nitazoxanide	3.04	10

3.6 Robustness

The method was robust against small, deliberate variations in flow rate (±0.2 mL/min) and mobile phase composition (±5% organic phase). System suitability parameters remained within acceptable limits.

Table 8: Robustness Summary

Parameter Modified	Ofloxacin Rt (min)	Nitazoxanide Rt (min)	Resolution	Tailing Factor
Flow ↑ (1.2 mL/min)	2.15	3.56	7.2	<2
Flow ↓ (0.8 mL/min)	2.68	4.41	8.3	<2
Organic ↑ (50%)	2.32	3.81	8.1	<2
Organic ↓ (40%)	2.57	4.02	8.6	<2

4. Conclusion

A robust and reliable RP-HPLC method was successfully developed and validated for the simultaneous estimation of Ofloxacin and Nitazoxanide in combined pharmaceutical dosage forms. The chromatographic conditions were optimized to achieve good resolution, peak symmetry, and minimal run time. The method demonstrated excellent linearity, accuracy, precision, robustness, and specificity, in accordance with ICH

Q2 (R1) guidelines. Low LOD and LOQ values confirmed the method's sensitivity. Overall, the developed RP-HPLC method is suitable for routine quality control and assay of Ofloxacin and Nitazoxanide in combined dosage formulations.

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