

Analytical Method Development and Validation of Nafithromycin by Reverse Phase HPLC

Kalyani Wajre*, Dr. Santosh Payghan, Prof. Amol Wadhve, Prof. Ashwini Pundkar.

1. Research scholar, Dept. of Pharmaceutical Quality Assurance, Rajesh Bhiyya Tope College of Pharmacy, Chh. Sambhajinagar.
2. Principal. Dept. of Pharmaceutical Sciences, Rajesh Bhiyya Tope College of Pharmacy, Chh. Sambhajinagar.
3. Professor, Dept. of Pharmaceutical Sciences, Rajesh Bhiyya Tope College of Pharmacy, Chh. Sambhajinagar.
4. Professor, Dept. of Pharmaceutical Sciences, Rajesh Bhiyya Tope College of Pharmacy, Chh. Sambhajinagar.

Email id: uionyou65@gmail.com

Abstract

Nafithromycin is India's first indigenous macrolide antibiotic, which belongs to the sub class of ketolides developed in India with support from BIRAC. It is a semi synthetic derivative of erythromycin, structurally featuring a tableted formulation and a thiadiazole-pyridine side chain, which enhances lung penetration. This innovation marks a milestone in pharmaceutical development and strengthens India's hand in the global fight against AMR. The drug targets community-acquired bacterial pneumonia (CABP), including drug-resistant infections, and is a major step in combating global antimicrobial resistance (AMR). Nafithromycin shows strong activity against a wide range of gram-positive bacteria, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and atypical pathogens such as *Mycoplasma pneumoniae*.

Key Words: Nafithromycin, community-acquired bacterial pneumonia (CABP); drug-resistant pathogens; RP-HPLC; antibiotic innovation; short-course therapy.

Introduction

Analytical method development occupies a central position in pharmaceutical quality assurance because every important decision concerning identity, purity, assay, dissolution, stability, and batch release depends on analytical evidence. A method is not merely a laboratory procedure; it is a control tool that links chemistry to product quality and regulatory confidence. For a new drug substance, the analytical scientist must identify a procedure that is scientifically sound, operationally simple, and fit for its intended purpose from development through commercial quality control. This requirement explains why chromatographic method development is treated as a planned scientific activity rather than as a trial-and-error exercise.

Rationale of The Study

- Nafithromycin is scientifically important because it represents a new antibacterial entity with clear relevance to respiratory infection management. New APIs require dedicated analytical understanding since assumptions based on older or structurally related drugs may not provide adequate selectivity or robustness. A thesis focused on nafithromycin by RP-HPLC is therefore justified as a scientific exercise in analyte-specific method development. It allows detailed consideration of molecular behavior, detector response, buffer choice, and chromatographic optimization in relation to a contemporary drug substance.
- The scientific rationale is strengthened by the currently available literature. Existing publications show that nafithromycin has been studied extensively from pharmacological and clinical standpoints, yet open routine assay literature remains limited compared with the drug's therapeutic significance.

MATERIALS

Essential materials for a nafithromycin RP-HPLC study include a qualified nafithromycin working standard or reference standard, HPLC-grade acetonitrile and methanol, purified water, sodium or potassium phosphate salts for buffer preparation, orthophosphoric acid for pH adjustment, representative placebo excipients, membrane filters, calibrated glassware, micropipettes, and weighing accessories. Reagent quality is important because impurities in solvents and buffers can destabilize the baseline, reduce sensitivity, or generate ghost peaks

Detector, diluent, and wavelength screening

Detector selection ordinarily begins with comparison of UV and PDA behavior because both are widely available in pharmaceutical laboratories. A PDA detector often offers practical advantages through spectral acquisition, improved peak purity assessment, and flexible wavelength selection.

Stationary phase and mobile phase screening strategy

Method screening usually begins on a conventional C18 stationary phase because it offers a broadly useful balance of retention and availability for drug substances of moderate to high hydrophobicity.

Buffer selection, pH control, flow rate, and temperature

For analytes with ionizable functionality, pH control is one of the most influential method variables. A suitable buffer helps maintain reproducible analyte ionization and reduces unwanted interactions that can broaden peaks or destabilize retention.

Standard preparation and stock solution design

A standard stock solution may be prepared by accurately weighing nafithromycin working standard and dissolving it in a suitable diluent to obtain a convenient concentration such as 1000 micrograms per millilitre.

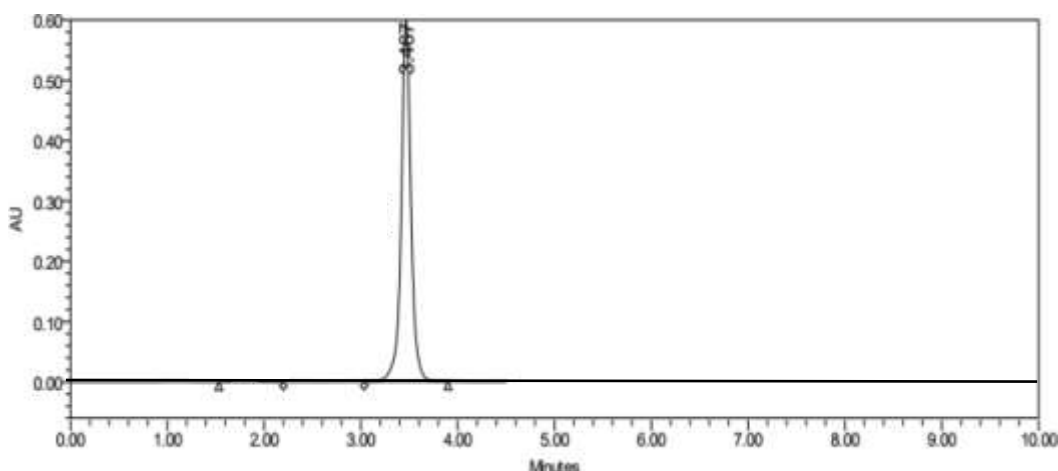
Linearity, Range, LOD, and LOQ studies

Linearity studies involve preparation of multiple calibration levels spanning the intended analytical range, followed by regression analysis of detector response versus concentration

Result and Discussion

Optimization of HPLC parameters

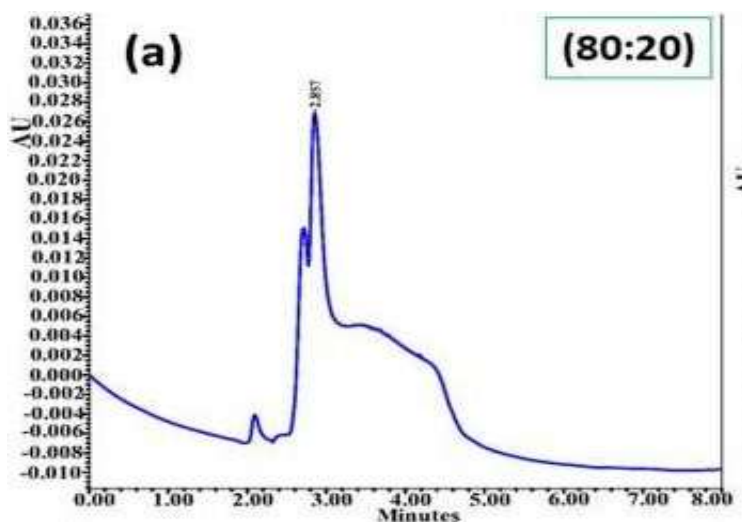
Since the Photodiode Array (PDA) detector offers several advantages over the conventional UV-Vis detector, the PDA was selected for chromatographic analysis of the standard mixture containing. The resulting chromatogram is presented in Fig. 1. Chromatographic separation of Nafithromycin using a PDA detector.



Investigation of mobile phase ratio

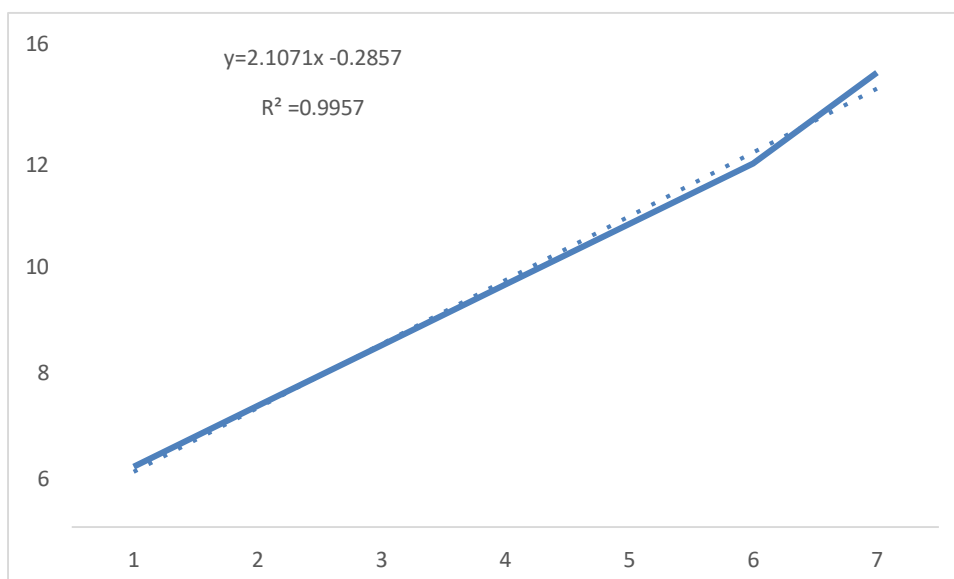
After identifying acetonitrile and phosphate buffer as the most suitable mobile phase components, different volume ratios of ACN:NaH₂PO₄ were tested (90:10, and 10:90, v/v) to determine the optimal composition for analysis. These experiments are presented in Fig. 3 and summarized in Table 2. Fig. 3. Chromatograms of Nafithromycin

obtained using different volume ratios of acetonitrile and phosphate buffer as the mobile phase: (a) 80:20. Chromatographic conditions: Agilent Zorbax Eclipse C18 column (250 × 4.6 mm, 5 μm) maintained at 35 °C; flow rate 0.8 mLmin⁻¹; injection volume 10 μL; detection using PDA detector.



Calibration curve Nafithromycin

Nafithromycin	
Concentration (ppm)	Peak area(mAu)
2	1902
4	4120
6	6731
8	9128
10	11723
12	14033
15	18013
$y=2.1071x-0.2857$	
$R^2=0.9957$	



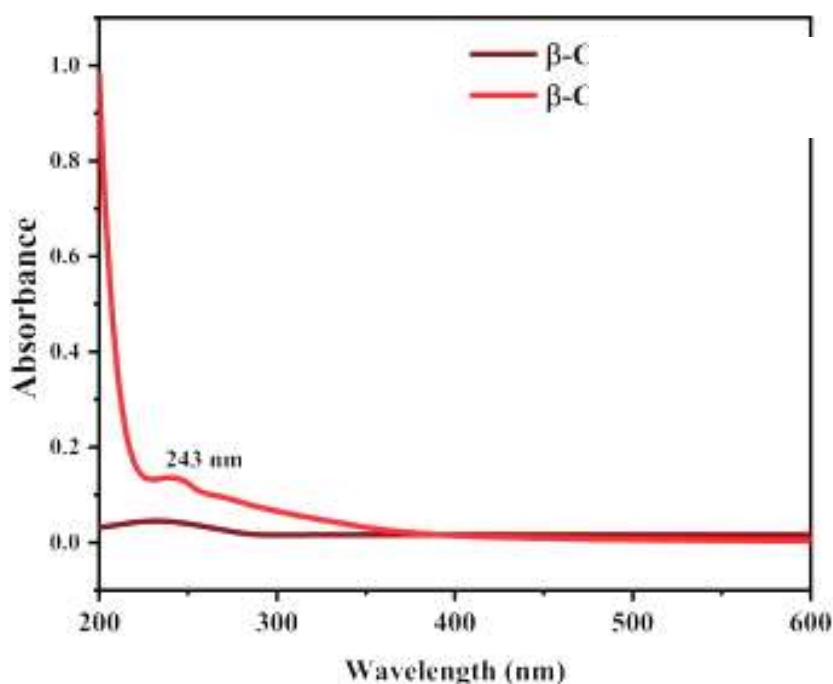
Recovery

To evaluate the recovery, the real sample S11 was first diluted by adding 7 mL of double-distilled water into a 100 mL volumetric flask and then filling up to the mark with the S11 solution. For the spiked sample, 2 mL of Nafithromycin (100 ppm) were added to a 100 mL volumetric flask, followed by dilution to the mark with the, which were 2, 2 and 3 $\mu\text{g mL}^{-1}$ spiked in the real sample. Both the diluted and spiked S11 samples were analyzed by HPLC. Based on the chromatographic results, calibration curves, and eqn (6), the recovery rates were calculated. The results are presented in Table 9 and Fig. 7.

Table 9. Recovery test results for Nafithromycin in real sample S11 (Standard addition method).

Analyte	Peak area	Peak area in spiked	Conc. In real sample	Conc. in spiked real sample	Recovery (R %)
Nafithromycin	4510	6964	4.045	6.040	99.75

Robustness



Summary and Conclusion

The present thesis was assembled to provide a complete pharmaceutical quality assurance document on analytical method development and validation of nafithromycin by reverse phase HPLC. The front chapters established the therapeutic importance of nafithromycin, the continuing value of RP-HPLC in routine pharmaceutical analysis, and the regulatory framework provided by ICH Q14 and Q2(R2). A detailed literature review then showed that nafithromycin has substantial support in medicinal chemistry, microbiology, pharmacokinetics, and clinical literature, but comparatively fewer open routine assay methods on conventional HPLC systems.

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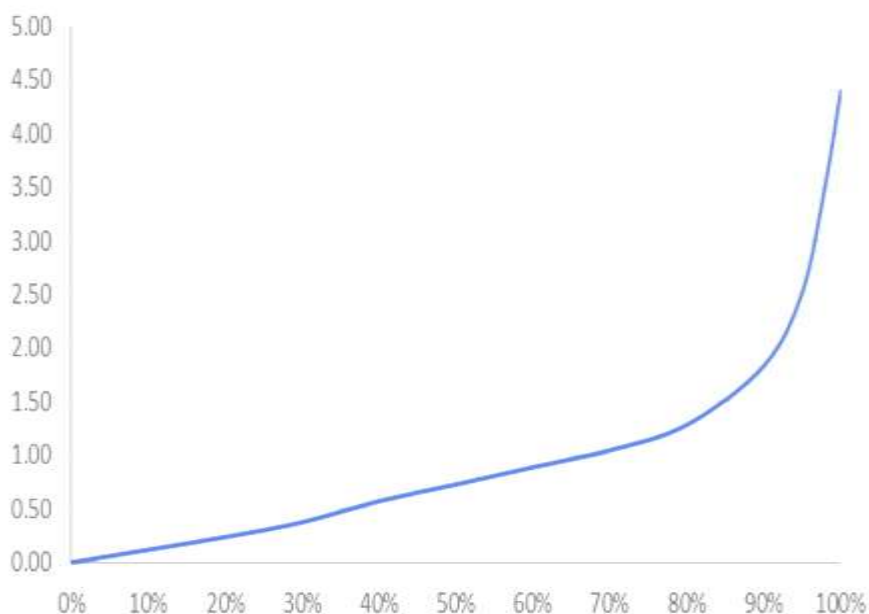


Fig. Chromatograms of Nafithromycin before