

Development and Validation of a Stability-Indicating UPLC Method for the Simultaneous Determination of Lazertinib and Three Nitroso Impurities

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ABSTRACT

Lazertinib is a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor used for the treatment of EGFR-mutated non-small cell lung cancer. The control of nitroso impurities associated with pharmaceutical drug substances is an important aspect of quality assessment because these compounds require sensitive and reliable analytical monitoring. The present study aimed to develop and validate a rapid stability-indicating ultra-performance liquid chromatographic (UPLC) method for the simultaneous determination of Lazertinib and three associated nitroso impurities, namely Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2 and Lazer Nitroso Impurity-3. Chromatographic separation was achieved using an Acquity UPLC BEH Phenyl column (2.1 × 100 mm, 1.7 µm) with acetonitrile and 0.1% triethylamine buffer adjusted to pH 2.5 with formic acid (40:60, v/v) as the mobile phase. The flow rate was 0.2 mL/min, injection volume was 5 µL, detection was performed at 257 nm, and the total run time was 6 min. The analytes were well separated with retention times of 1.412, 2.675, 3.141 and 4.122 min for Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, Lazer Nitroso Impurity-3 and Lazertinib, respectively. The corresponding resolution values were 6.34, 2.39 and 4.95. The method demonstrated linearity over 62.5–375 µg/mL for Lazertinib and 2.5–15 µg/mL for each nitroso impurity, with correlation coefficients of 0.99993, 0.99983, 0.99994 and 0.99995, respectively. Accuracy was demonstrated by recoveries of 99.4–101.0%, while precision results showed %RSD values below 1%. The LOD and LOQ were 0.30 and 1.00 µg/mL, respectively, for Lazertinib and all three nitroso impurities. The method remained unaffected by deliberate variations in flow rate and mobile-phase composition. Forced-degradation studies demonstrated degradation under acidic, alkaline, oxidative and thermal conditions, whereas Lazertinib showed comparatively greater stability under reductive, photolytic and hydrolytic conditions. The developed UPLC procedure therefore provides a rapid and reliable chromatographic approach for the determination of Lazertinib and its associated nitroso impurities and can be applied for stability assessment and impurity profiling of Lazertinib.

Keywords: Lazertinib; nitroso impurities; UPLC; impurity profiling; stability-indicating method; pharmaceutical analysis.

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INTRODUCTION

Lazertinib is a third-generation epidermal growth factor receptor tyrosine kinase inhibitor developed for the treatment of non-small cell lung cancer (NSCLC) associated with specific activating and resistance

mutations in the EGFR gene [1]. The drug is used clinically in combination with amivantamab for adults with locally advanced or metastatic NSCLC harboring EGFR exon 19 deletions or exon 21 L858R

substitution mutations. Unlike earlier generations of EGFR tyrosine kinase inhibitors, Lazertinib was developed with activity against mutant EGFR, including the T790M resistance mutation [2]. Its pharmacological activity is associated with irreversible inhibition of EGFR through covalent interaction with the Cys797 residue located within the ATP-binding site of the kinase domain. This inhibition suppresses downstream signaling pathways, including EGFR, AKT and ERK phosphorylation, ultimately reducing proliferation and promoting apoptosis of EGFR-mutant cancer cells [3].

Chemically, Lazertinib is designated as N-[5-[[4-[4-[(dimethylamino)methyl]-3-phenylpyrazol-1-yl]pyrimidin-2-yl]amino]-4-methoxy-2-morpholin-4-ylphenyl]prop-2-enamide (**Fig. 1**). It has the molecular formula $C_{30}H_{34}N_8O_3$ and a molecular weight of 554.655 g/mol. The molecule incorporates several nitrogen-containing heterocyclic and amino functionalities, including pyrimidine, pyrazole and morpholine structural units. Such structural characteristics are relevant from an analytical perspective because the parent drug and structurally related process- or degradation-derived components may exhibit different chromatographic interactions and require adequate selectivity for their simultaneous determination [4].

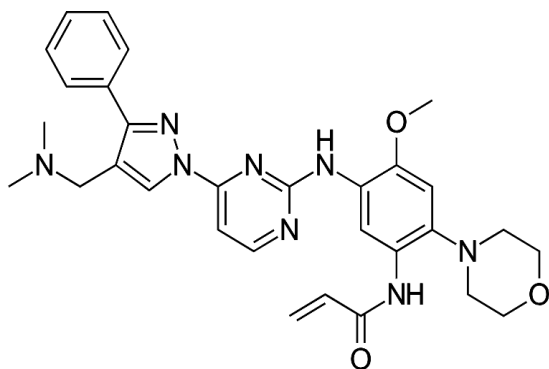


Fig. 1. Chemical structure of Lazertinib

The quality assessment of pharmaceutical drug substances involves not only determination of the active pharmaceutical ingredient (API) but also comprehensive monitoring of process-related impurities, degradation products and potentially hazardous impurities. Among these, N-nitroso compounds have received particular analytical attention because their formation can occur through nitrosation pathways involving suitable amine-containing structures [5]. Consequently, sensitive and selective analytical procedures are required when

nitroso impurities are identified or considered as potential impurities associated with a pharmaceutical substance. An analytical method intended for such an application should provide adequate chromatographic resolution between the API and individual nitroso impurities while maintaining suitable sensitivity, precision and reproducibility [6].

In the present study, three Lazertinib-associated nitroso impurities were investigated, designated Lazer Nitroso Impurity-1 (**Fig. 2a**), Lazer Nitroso Impurity-2 (**Fig. 2b**) and Lazer Nitroso Impurity-3 (**Fig. 2c**). According to the experimental source data, Lazer Nitroso Impurity-1 has a molecular formula of $C_{15}H_{15}ClN_6O_5$ and molecular weight of 394.8 g/mol. Lazer Nitroso Impurity-2 has a molecular formula of $C_{25}H_{22}N_8O_6$ and molecular weight of 530.5 g/mol, whereas Lazer Nitroso Impurity-3 has a molecular formula of $C_{27}H_{29}N_9O_5$ and molecular weight of 559.6 g/mol. The structural differences between these impurities and Lazertinib provide an analytical basis for their chromatographic discrimination and simultaneous monitoring.

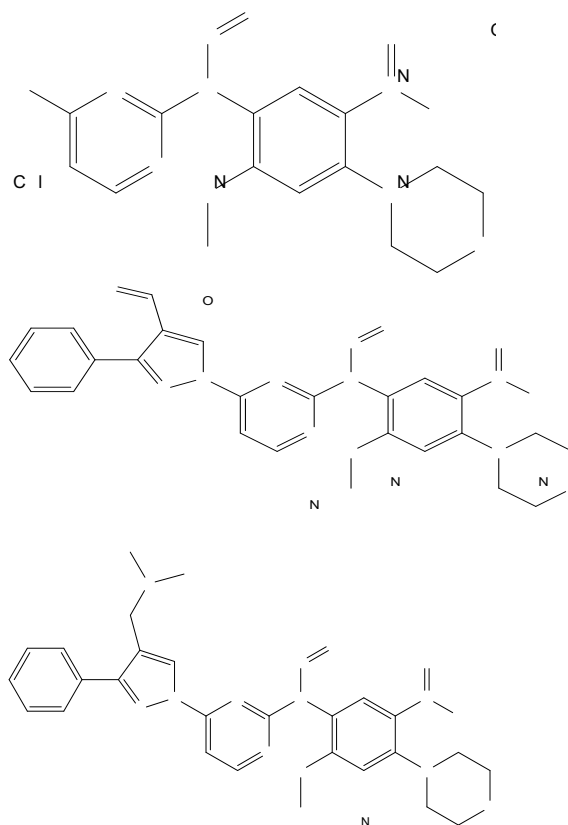


Fig. 2. Chemical structures of (a) Lazer Nitroso Impurity-1, (b) Lazer Nitroso Impurity-2, and (c) Lazer Nitroso Impurity-3.

The analytical literature available for Lazertinib has primarily focused on determination of the drug in biological matrices and pharmaceutical formulations. An LC–MS/MS method has been reported for quantification of Lazertinib in rat plasma following solidified floating organic drop microextraction. In that study, chromatographic separation was performed using a reversed-phase column with acetonitrile and 0.1% formic acid in water, and the method was subsequently applied to pharmacokinetic evaluation [7]. Another reported procedure employed solid-phase extraction followed by reversed-phase HPLC for determination of Lazertinib in human plasma for therapeutic drug-monitoring applications [8].

Although these methods demonstrate the chromatographic determination of Lazertinib in different analytical matrices, the experimental objective of the present work was specifically directed toward simultaneous separation of Lazertinib and its three associated nitroso impurities together with evaluation of degradation-related components. This requires greater emphasis on chromatographic selectivity than an assay procedure intended solely for determination of the API. In particular, the analytical method should provide sufficient resolution of closely eluting impurity peaks, acceptable peak symmetry, adequate sensitivity for low-level impurities and reproducible performance following deliberate changes in chromatographic parameters [9].

Ultra-performance liquid chromatography (UPLC) provides an appropriate platform for this purpose because the use of small-particle stationary phases permits efficient chromatographic separation with relatively short analysis times and reduced mobile-phase consumption [10]. During development of the present method, different chromatographic conditions were investigated, including changes in stationary phase, mobile-phase composition and flow rate. Initial trials using an X-Bridge C18 column and different mobile-phase compositions provided separation of the investigated components but did not consistently provide the desired chromatographic performance. Subsequent optimization using an Acquity UPLC BEH Phenyl column provided improved separation of all four principal chromatographic components [11].

Thus, the present study was undertaken to develop a rapid, selective, sensitive and stability-indicating UPLC method for simultaneous determination of Lazertinib and three associated nitroso impurities. The developed method was systematically evaluated for chromatographic performance, specificity, linearity, accuracy, precision, sensitivity, robustness and forced-

degradation behavior. The resulting analytical procedure provides a short-run chromatographic approach for impurity profiling of Lazertinib and assessment of its behavior under different stress conditions.

MATERIALS AND METHODS

Chemicals and reagents

Lazertinib working standard and the corresponding reference standards of Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 were procured from Merck (India) and used for method development and validation. Acetonitrile, triethylamine (TEA), and HPLC-grade water were also procured from Merck (India), Mumbai, India. The pharmaceutical product Lazcluze® (80 mg Lazertinib) was procured from Janssen Pharmaceuticals, Mumbai, India. Unless otherwise specified, all solvents and reagents used for chromatographic method development, sample preparation, method optimization, and validation were of analytical reagent or UPLC grade. All materials were used as received without further purification.

Preparation of standard solutions

Lazertinib standard stock solution

An accurately weighed quantity of 25 mg of Lazertinib working standard was transferred into a 10 mL volumetric flask. Diluent was added and the solution was sonicated to ensure complete dissolution. The volume was subsequently made up to the mark with the same diluent to obtain the Lazertinib stock solution (2500 µg/mL) [12].

Nitroso impurity stock solutions

Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2 and Lazer Nitroso Impurity-3 were accurately weighed individually at 10 mg each and transferred into 10 mL volumetric flasks. Approximately 7 mL of diluent was added and the solutions were sonicated for 30 min. The volume was then made up to the mark with diluent. Each solution was further diluted by transferring 1 mL into a 10 mL volumetric flask and making up to volume with diluent to obtain the working impurity stock solutions [13].

Standard mixture

A mixed standard solution was prepared by transferring 1 mL each of the Lazertinib and three nitroso impurity stock solutions into a 10 mL volumetric flask and diluting to volume with the diluent. The resulting solution was used for chromatographic optimization and system suitability assessment.

Preparation of Lazertinib sample solution

An accurately weighed quantity of 56 mg of Lazertinib sample was transferred into a 10 mL volumetric flask. Diluent was added and the sample was sonicated to achieve complete dissolution, followed by dilution to volume with the same diluent. For impurity evaluation, 1 mL of the impurity stock solution and 1 mL of the Lazertinib sample stock solution were further diluted to 10 mL with diluent. The resulting solution was passed through a 0.22 μm nylon syringe filter before injection into the UPLC system.

UPLC instrumentation and optimized chromatographic conditions

Chromatographic analysis was performed using an ACQUITY™ UPLC system (Waters Corporation, Milford, MA, USA) equipped with a photodiode-array (PDA) detector. Instrument control, chromatographic data acquisition, peak integration, and quantitative data processing were performed using Empower™ 2.0 chromatography software (Waters Corporation). Separation of Lazertinib and Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 was achieved using an ACQUITY UPLC BEH Phenyl column (2.1 $\times$ 100 mm, 1.7 μm ; Waters Corporation). The column was selected based on its chromatographic performance during method development, particularly with respect to separation efficiency, peak shape, and resolution of Lazertinib and its nitroso impurities. The optimized mobile phase consisted of acetonitrile and 0.1% (v/v) triethylamine (TEA) buffer adjusted to pH 2.5 with formic acid (40:60, v/v). Chromatographic separation was performed under isocratic elution at a flow rate of 0.2 mL min⁻¹. The PDA detector was set at 257 nm for simultaneous monitoring of Lazertinib and its nitroso impurities. The injection volume was 5 μL , and the total chromatographic run time was 6 min [14].

UPLC method development

A reversed-phase UPLC approach was adopted for the simultaneous separation of Lazertinib and its three associated nitroso impurities, namely Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3. Method development was performed systematically by evaluating the chromatographic mode, stationary phase, mobile-phase composition, aqueous-phase pH, flow rate, detection wavelength, and injection volume. The selection of chromatographic conditions was primarily guided by the ability to obtain adequate separation of the nitroso impurities from Lazertinib, satisfactory peak shape, consistent detector response, and a short analysis time. The detection wavelength was established using PDA spectral scanning of Lazertinib and the individual nitroso impurity standards. Based on the spectral response and the suitability for simultaneous monitoring of all investigated components, 257 nm was selected as the working

detection wavelength. Different reversed-phase stationary phases and mobile-phase compositions were subsequently investigated to achieve satisfactory chromatographic separation [15].

Initial chromatographic trials were performed using an X-Bridge C18 column with different mobile-phase conditions. An acetonitrile-based mobile phase containing an acidic aqueous component was initially evaluated; however, the obtained chromatographic performance did not provide the desired retention and separation characteristics within the targeted analysis time. Further optimization was therefore performed using an ACQUITY UPLC BEH Phenyl column (2.1 $\times$ 100 mm, 1.7 μm). The BEH Phenyl stationary phase provided improved chromatographic selectivity toward Lazertinib and its nitroso impurities and was therefore selected for subsequent optimization. The composition and pH of the aqueous phase were then systematically adjusted to improve peak shape and separation efficiency. A 0.1% (v/v) triethylamine (TEA) buffer adjusted to pH 2.5 with formic acid was selected as the aqueous component. The proportion of acetonitrile and aqueous buffer was optimized to obtain satisfactory separation while maintaining a short chromatographic analysis time. The final mobile-phase composition was established as acetonitrile:0.1% TEA buffer adjusted to pH 2.5 with formic acid (40:60, v/v). The mobile phase was delivered under isocratic conditions at a flow rate of 0.2 mL min⁻¹.

Other chromatographic parameters were optimized to ensure reproducible and efficient analysis. The column was maintained at ambient temperature, and an injection volume of 5 μL was selected. Detection was performed at 257 nm, and the total chromatographic run time was established as 6 min. The optimized conditions provided satisfactory chromatographic separation of Lazertinib from Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 and were subsequently used for method validation [16, 17].

Method validation

The developed UPLC method was validated in accordance with ICH Q2(R1) guidelines with respect to specificity, linearity and range, accuracy, system precision, method precision, intermediate precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and forced degradation/stability-indicating capability. All validation experiments were performed using the optimized chromatographic conditions described above [18, 19].

Specificity

Specificity was evaluated to demonstrate the ability of the method to determine Lazertinib and its three nitroso impurities without interference from other components potentially present in the analytical

matrix. Separate chromatographic injections of the blank, placebo, standard solution, and sample solution were performed. In addition, the sample was spiked with Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 and analyzed under the optimized chromatographic conditions. The resulting chromatograms were examined for any interfering peaks at the chromatographic regions corresponding to Lazertinib and the three nitroso impurities.

Linearity and range

Linearity was evaluated over six concentration levels corresponding to 25, 50, 75, 100, 125, and 150% of the working concentration. A Lazertinib stock solution was prepared by accurately weighing 25 mg of Lazertinib working standard into a 10 mL volumetric flask, dissolving it in diluent, and making the volume up to the mark to obtain a concentration of 2500 $\mu\text{g mL}^{-1}$. For the nitroso impurities, 10 mg each of Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 were accurately weighed and transferred into a 10 mL volumetric flask. The standards were dissolved in diluent and diluted to volume. Subsequently, 1.0 mL of this solution was transferred into a 10 mL volumetric flask and diluted to the mark with diluent to obtain an impurity stock solution containing 100 $\mu\text{g mL}^{-1}$ of each impurity.

The six linearity levels for Lazertinib were prepared at 62.5, 125.0, 187.5, 250.0, 312.5, and 375.0 $\mu\text{g mL}^{-1}$, while the corresponding concentrations of each nitroso impurity were 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 $\mu\text{g mL}^{-1}$, respectively. Appropriate aliquots of the Lazertinib and impurity stock solutions were transferred into 10 mL volumetric flasks and diluted to volume with diluent. Each concentration level was analyzed using the optimized UPLC method, and the chromatographic peak area was plotted against the corresponding analyte concentration. Linear regression analysis was performed to obtain the slope, intercept, and correlation coefficient for Lazertinib and each nitroso impurity. A correlation coefficient of not less than 0.999 was used as the predefined acceptance criterion for linearity [20].

Accuracy

Accuracy was assessed by a recovery study performed at 80, 100, and 120% levels. For preparation of the Lazertinib standard stock solution, 25 mg of Lazertinib working standard was accurately weighed, transferred into a 10 mL volumetric flask, dissolved in diluent, and diluted to volume to obtain a concentration of 2500 $\mu\text{g mL}^{-1}$. The sample stock solution was prepared by accurately weighing 56 mg of Lazertinib sample into a 10 mL volumetric flask. Diluent was added, the solution was sonicated to ensure complete dissolution, and the volume was made up to the mark with the same diluent.

The impurity stock solution was prepared by accurately weighing 10 mg each of Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 into a 10 mL volumetric flask. The impurities were dissolved using diluent and diluted to volume. A 1.0 mL aliquot of the resulting solution was subsequently diluted to 10 mL with diluent. For the 80% accuracy level, 1.0 mL of sample stock solution, 1.0 mL of impurity stock solution, and 0.8 mL of Lazertinib standard stock solution were transferred into a 10 mL volumetric flask. The solution was sonicated for approximately 20 min and diluted to volume with diluent. The resulting concentration corresponded to approximately 450 $\mu\text{g mL}^{-1}$ Lazertinib and 10 $\mu\text{g mL}^{-1}$ of each nitroso impurity. For the 100% accuracy level, 1.0 mL of sample stock solution, 1.0 mL of impurity stock solution, and 1.0 mL of Lazertinib standard stock solution were combined in a 10 mL volumetric flask, sonicated for 20 min, and diluted to volume, providing approximately 500 $\mu\text{g mL}^{-1}$ Lazertinib and 10 $\mu\text{g mL}^{-1}$ of each nitroso impurity. For the 120% accuracy level, 1.0 mL of sample stock solution, 1.0 mL of impurity stock solution, and 1.2 mL of Lazertinib standard stock solution were transferred into a 10 mL volumetric flask, sonicated for 20 min, and diluted to the mark, corresponding to approximately 550 $\mu\text{g mL}^{-1}$ Lazertinib and 10 $\mu\text{g mL}^{-1}$ of each nitroso impurity. The prepared solutions were analyzed under the optimized chromatographic conditions, and accuracy was expressed as percentage recovery of Lazertinib and each impurity. The predefined acceptance range for recovery was 98–102% [21].

System precision

System precision was evaluated using six replicate injections of a standard solution containing 250 $\mu\text{g mL}^{-1}$ Lazertinib and 10 $\mu\text{g mL}^{-1}$ each of Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3. The chromatographic peak areas obtained from the six injections were recorded for each analyte. The mean, standard deviation, and percentage relative standard deviation (%RSD) of the peak areas were calculated to assess repeatability of the chromatographic system.

Method precision

Method precision was assessed by preparing and analyzing six independent sample preparations under the same experimental conditions. Each preparation was injected using the validated chromatographic method, and the peak areas of Lazertinib and the three nitroso impurities were recorded. The mean, standard deviation, and %RSD of the responses obtained from the six preparations were calculated. A %RSD not greater than 2.0% was considered acceptable for method precision.

Intermediate precision

Intermediate precision was evaluated to determine the reproducibility of the analytical procedure under normal laboratory variations. The analysis was repeated using different analysts and different instruments within the same laboratory facility. Six independent determinations were performed, and the chromatographic responses of Lazertinib, Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 were recorded. Intermediate precision was expressed as the mean, standard deviation, and %RSD of the analytical responses [22].

Limit of detection and limit of quantification

The sensitivity of the method was evaluated by determining the LOD and LOQ for Lazertinib and each of the three nitroso impurities. The LOD was established at the concentration that produced a signal-to-noise ratio of approximately 3:1, whereas the LOQ was established at a signal-to-noise ratio of approximately 10:1. Appropriate low-concentration solutions of Lazertinib and each nitroso impurity were prepared and analyzed under the optimized chromatographic conditions to verify the corresponding signal-to-noise responses.

Robustness

Robustness was evaluated by deliberately introducing small variations in selected chromatographic parameters while maintaining the remaining experimental conditions unchanged. The influence of flow rate and mobile-phase composition was specifically examined. The nominal flow rate of 0.20 mL min⁻¹ was varied to 0.18 and 0.22 mL min⁻¹. Similarly, the optimized mobile-phase composition of acetonitrile:0.1% TEA buffer adjusted to pH 2.5 with formic acid (40:60, v/v) was altered to 36:64 (v/v) to represent a lower organic proportion and 44:56 (v/v) to represent a higher organic proportion. The analytical solutions were analyzed under each modified condition, and the chromatographic responses were compared with those obtained under the nominal conditions. Robustness was assessed principally on the basis of the variation in analytical response expressed as %RSD.

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability-indicating capability of the developed UPLC method and to investigate the behavior of Lazertinib under different stress conditions. Lazertinib samples were subjected to acidic, alkaline, oxidative, reductive, thermal, photolytic, and hydrolytic stress conditions. Following stress treatment, the solutions were appropriately diluted, spiked with the impurity stock solution where specified, and analyzed using the optimized UPLC procedure. For preparation of the impurity stock solution used in the degradation studies, 10 mg each of Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3

were transferred into a 10 mL volumetric flask, dissolved in diluent, and diluted to volume. Subsequently, 1.0 mL of this solution was diluted to 10 mL with diluent [23, 24].

Acid degradation

For acid degradation, 56 mg of Lazertinib sample was transferred into a 10 mL volumetric flask and treated with 1.0 mL of 1 N HCl at room temperature for 30 min. The stressed solution was neutralized with 1.0 mL of 1 N NaOH, sonicated for approximately 20 min, and diluted to volume with diluent. Thereafter, 1.0 mL of the stressed solution and 1.0 mL of impurity stock solution were transferred into another 10 mL volumetric flask and diluted to volume. The final solution was filtered through a 0.22 µm nylon syringe filter before chromatographic analysis.

Alkaline degradation

For alkaline degradation, 56 mg of Lazertinib sample was treated with 1.0 mL of 1 N NaOH at room temperature for 30 min. The stressed sample was neutralized with 1.0 mL of 1 N HCl, sonicated for approximately 20 min, and diluted to volume with diluent. A 1.0 mL aliquot of the stressed solution and 1.0 mL of impurity stock solution were further diluted to 10 mL and filtered through a 0.22 µm nylon syringe filter.

Oxidative degradation

For oxidative degradation, 56 mg of Lazertinib sample was treated with 1.0 mL of 10% H₂O₂ and maintained at room temperature for 30 min. The mixture was sonicated for 20 min and diluted to volume with diluent. Subsequently, 1.0 mL of the stressed solution and 1.0 mL of impurity stock solution were diluted to 10 mL and filtered through a 0.22 µm nylon syringe filter.

Reductive degradation

For reductive degradation, 56 mg of Lazertinib sample was exposed to 1.0 mL of 10% sodium bisulfite solution at room temperature for 30 min. The sample was sonicated for approximately 20 min and diluted to volume. A 1.0 mL aliquot of the stressed sample solution was combined with 1.0 mL of impurity stock solution, diluted to 10 mL, and filtered before analysis.

Thermal degradation

For thermal degradation, 56 mg of Lazertinib sample was exposed to 105 °C for 6 h. After thermal treatment, 56 mg of the exposed sample was transferred into a 10 mL volumetric flask, approximately 7 mL of diluent was added, and the solution was sonicated until dissolved and diluted to volume. An aliquot of the resulting solution was further diluted with diluent and combined with the impurity stock solution before chromatographic analysis.

Photolytic degradation

For photolytic degradation, 56 mg of Lazertinib sample was exposed for 3 days to light providing 1.2 million lux hours and an integrated near-ultraviolet energy of not less than 200 Wh m⁻². The exposed material was transferred into a 10 mL volumetric flask, approximately 7 mL of diluent was added, and the mixture was sonicated and diluted to volume. The stressed sample was subsequently diluted, combined with the impurity stock solution, filtered through a 0.22 µm nylon syringe filter, and transferred to chromatographic vials.

Hydrolytic degradation

For hydrolytic degradation, 56 mg of Lazertinib sample was treated with 1.0 mL of HPLC-grade water at room temperature for 30 min. The mixture was sonicated for approximately 20 min and diluted to volume with diluent. A 1.0 mL aliquot of the stressed solution was combined with 1.0 mL of impurity stock solution, diluted to 10 mL, and filtered through a 0.22 µm nylon syringe filter prior to analysis. All stressed samples were analyzed using the optimized UPLC method to assess the separation of Lazertinib, the three specified nitroso impurities, and stress-induced degradation components, thereby evaluating the stability-indicating performance of the analytical procedure.

RESULTS AND DISCUSSION

Development and optimization of the UPLC method

The chromatographic method was developed to achieve efficient and reproducible separation of Lazertinib from Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 within a short analysis time. During method development, the stationary phase, mobile-phase composition, aqueous-phase pH, flow rate, and injection volume were investigated. PDA scanning of the analytes indicated a suitable common detection wavelength of 257 nm, which was therefore selected for subsequent chromatographic experiments. Initial experiments were performed on an X-Bridge C18 column (50 × 4.6 mm, 2.1 µm). In the first trial, acetonitrile and 0.1% trifluoroacetic acid (20:80, v/v) were used as the mobile phase at a flow rate of 0.3 mL min⁻¹. Although all four components were detected, the chromatographic performance did not meet the intended system-suitability requirements. Increasing the organic-phase proportion to 40% improved the separation; however, the resulting chromatographic profile was not considered suitable because the retention characteristics were not within the targeted limits.

Further optimization was performed by replacing the acidic mobile phase with 0.1% TEA buffer adjusted to pH 2.5 with formic acid. Using the X-Bridge C18 column with this aqueous phase improved

chromatographic behavior, but adequate resolution between the components was still not consistently achieved. Consequently, a phenyl-based stationary phase was investigated to exploit its different selectivity toward Lazertinib and the nitroso impurities. An ACQUITY UPLC BEH Phenyl column was subsequently evaluated. Using a 2.1 × 50 mm, 1.7 µm column with acetonitrile:0.1% TEA buffer (pH 2.5 adjusted with formic acid) at 30:70 (v/v), separation of the analytes was improved; however, the chromatographic profile showed insufficient resolution between some adjacent components. Increasing the acetonitrile proportion to 40% improved the overall chromatographic response and separation characteristics, although the response of the peaks was relatively high and further optimization was considered necessary.

The column length was subsequently increased to 2.1 × 100 mm, while maintaining the optimized mobile-phase composition of acetonitrile:0.1% TEA buffer, pH 2.5 adjusted with formic acid (40:60, v/v). A flow rate of 0.2 mL min⁻¹, injection volume of 5 µL, ambient column temperature, and detection at 257 nm were employed. Under these conditions, all three nitroso impurities were adequately separated from Lazertinib, with resolution values of 6.34, 2.39, and 4.95 between successive chromatographic peaks. The observed peak-tailing factors ranged from 0.87 to 1.18, while the USP plate counts ranged from 5257 to 12,546, indicating satisfactory chromatographic efficiency (**Fig. 3**). The method was therefore considered suitable for subsequent validation. The optimized chromatographic conditions are summarized in **Table 1**.

Table 1. Optimized chromatographic conditions

Parameter	Chromatographic condition
Movable phase	Acetonitrile and 0.1% TEA of pH-2.5 adjusted with formic acid (40+60)
Column	Acquity UPLC BEH Phenyl (2.1x100mm, 1.7µm)
Rate of flow	0.2 ml/min
Column temperature	Ambient
Wavelength	257 nm
Volume of injection	5 µL
Period of run	6min

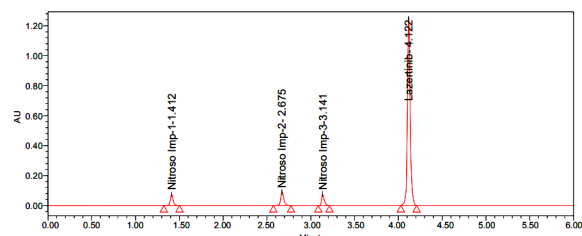


Fig. 3. Representative chromatogram obtained using the optimized UPLC method for simultaneous determination of Lazertinib and its three nitroso impurities.

Method validation

Specificity

Specificity was evaluated by comparing chromatograms obtained from the blank, placebo, standard, and sample solutions. The chromatographic evaluation demonstrated that Lazertinib and the three specified nitroso impurities could be monitored without apparent interference from the blank or formulation matrix at their respective chromatographic positions. The sample spiked with the three nitroso impurity standards also demonstrated their individual chromatographic responses, confirming that the developed procedure was capable of distinguishing the investigated impurities from Lazertinib. The satisfactory chromatographic separation observed during method development, together with the absence of significant interference in the blank and placebo chromatograms, supported the specificity of the developed UPLC procedure for simultaneous determination of Lazertinib and its three nitroso impurities (**Fig. 4**).

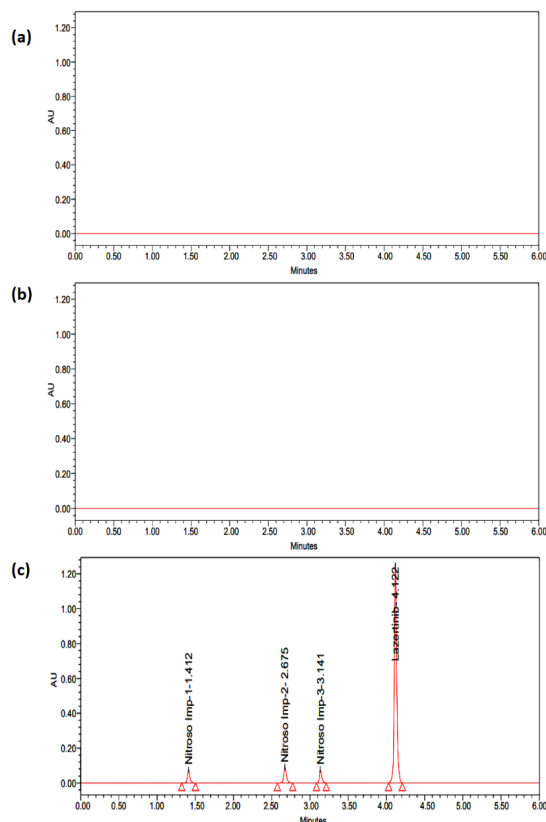


Fig. 4. Specificity assessment of the developed UPLC method using chromatograms of (a) blank, (b) placebo, and (c) Lazertinib sample spiked with Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3.

Linearity and range

The linearity of the developed method was investigated over six concentration levels. Lazertinib exhibited a linear response over the concentration range of 62.5–375.0 $\mu\text{g mL}^{-1}$, while each of the three nitroso impurities showed linearity over 2.5–15.0 $\mu\text{g mL}^{-1}$. For Lazertinib, the measured peak areas increased proportionally with concentration, and linear regression produced a slope of 60,142.40 and an intercept of 60,603.71, with a correlation coefficient of 0.99993. Lazer Nitroso Impurity-1 showed a slope of 8920.87, an intercept of 392.61, and a correlation coefficient of 0.99983. For Lazer Nitroso Impurity-2, the slope and intercept were 11,918.21 and 905.82, respectively, with a correlation coefficient of 0.99994. Lazer Nitroso Impurity-3 exhibited a slope of 9247.07, an intercept of 285.68, and a correlation coefficient of 0.99995. The corresponding coefficients of determination were approximately 0.99986, 0.99965, 0.99988, and 0.99990 for Lazertinib, Impurity-1, Impurity-2, and Impurity-3, respectively. Thus, all investigated components demonstrated an excellent linear relationship between concentration and

chromatographic peak area across the evaluated ranges (Fig. 5).

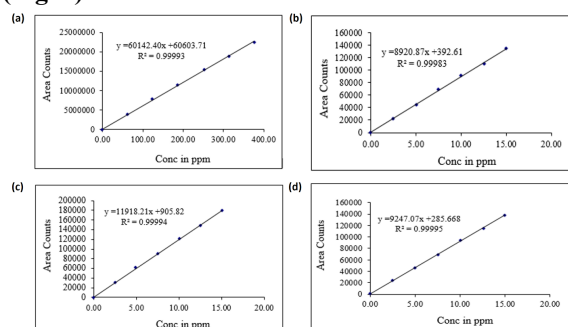


Fig. 5. Calibration curves for (a) Lazertinib, (b) Lazer Nitroso Impurity-1, (c) Lazer Nitroso Impurity-2, and (d) Lazer Nitroso Impurity-3 over the respective validated concentration ranges.

Accuracy

The accuracy of the analytical procedure was established by recovery experiments at 80, 100, and 120% concentration levels. The recovery results demonstrated close agreement between the added and measured concentrations for both Lazertinib and the three nitroso impurities. For Lazertinib, the percentage recoveries were 99.8, 100.1, and 99.5% at the 80, 100, and 120% levels, respectively. Lazer Nitroso Impurity-1 showed recoveries of 100.0, 100.1, and 100.1%, while Lazer Nitroso Impurity-2 showed recoveries of 99.4, 101.0, and 100.1% at the corresponding levels. Lazer Nitroso Impurity-3 exhibited recoveries of 99.8, 99.9, and 100.0%, respectively. All recovery values were within the predefined acceptance range of 98–102%, demonstrating the satisfactory accuracy of the developed procedure for quantitative determination of Lazertinib and its associated nitroso impurities (Table 2).

Table 2. Accuracy and percentage recovery results for Lazertinib and the three nitroso impurities at the evaluated concentration levels.

Quantity spiked	% Regeneration			
	Lazertinib	Lazer Nitroso Imp-1	Lazer Nitroso Imp-2	Lazer Nitroso Imp-3
80 percent	99.8	100.0	99.4	99.8
100 percent	100.1	100.1	101.0	99.9
120 percent	99.5	100.1	100.1	100.0

Precision

System precision

System precision was evaluated using six replicate injections of a solution containing 250 $\mu\text{g mL}^{-1}$ Lazertinib and 10 $\mu\text{g mL}^{-1}$ of each nitroso impurity. The %RSD values of the chromatographic peak areas were 0.54% for Lazertinib, 0.26% for Lazer Nitroso Impurity-1, 0.95% for Lazer Nitroso Impurity-2, and 0.13% for Lazer Nitroso Impurity-3. The low %RSD values demonstrated good repeatability of the chromatographic system and indicated consistent detector response and injection performance throughout the replicate analyses.

Method precision

Method precision was evaluated using six independently prepared sample solutions. The %RSD values obtained for Lazertinib, Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3 were 0.55, 0.23, 1.00, and 0.23%, respectively. The low variability among independently prepared sample solutions, with all %RSD values remaining below the predefined 2% criterion, confirmed the repeatability of the complete analytical procedure, including sample preparation and chromatographic measurement.

Intermediate precision

Intermediate precision was evaluated under changed analytical conditions involving different analysts and instruments within the same laboratory. The resulting %RSD values were 0.49% for Lazertinib, 0.26% for Lazer Nitroso Impurity-1, 0.53% for Lazer Nitroso Impurity-2, and 0.31% for Lazer Nitroso Impurity-3. The observed low variability demonstrated that the method maintained consistent analytical performance when subjected to normal within-laboratory variations. These results further supported the reproducibility of the developed UPLC procedure (Table 3 & Fig 5).

Table 3. Precision results for Lazertinib and the three nitroso impurities

Analyte	Concentration ($\mu\text{g/mL}$)	System precision	Sample precision	Method precision	Intermediate precision	Inte rme diat e prec isio n %R SD
Lazertinib	250	15, 207, 93 $\pm$ 81, 787.38	0.54	15, 257, 70 $\pm$ 4, 247.39	0.5	15.1, 99.7, 02 $\pm$ 74.2, 31.5, 54

Development and Validation of a Stability-Indicating UPLC Method for the Simultaneous Determination of Lazertinib and Three Nitroso Impurities

La zer Nit roso Im pur ity- 1	10	90, 524 ± 239 .28 8	0.2 6 205 .91 4	90, 549 ± 205 .91 4	0.2 3 841	90,4 84 ± 238. 841	0.26
La zer Nit roso Im pur ity- 2	10	121 ,94 4 ± 1,1 58. 806	0.9 5 1,2 18. 490	121 ,56 2 ± 1,2 18. 490	1.0 0 637. 331	121, 141 ± 637. 331	0.53
La zer Nit roso Im pur ity- 3	10	93, 618 ± 123 .11 2	0.1 3 216 .15 1	93, 606 ± 216 .15 1	0.2 3 040	93,4 95 ± 294. 040	0.31

Sensitivity

The sensitivity of the method was evaluated by determination of the LOD and LOQ for Lazertinib and the three nitroso impurities. The LOD was 0.30 µg mL⁻¹ for Lazertinib and each of the three impurities, corresponding to a signal-to-noise ratio of approximately 3:1. The LOQ was 1.00 µg mL⁻¹ for all four components, corresponding to a signal-to-noise ratio of approximately 10:1. The comparable sensitivity obtained for Lazertinib and each nitroso impurity demonstrated that the developed procedure was capable of detecting and quantifying the investigated components at low concentration levels. The results are summarized in **Table 4**.

Table 4. Limits of detection and quantification for Lazertinib and Lazer Nitroso Impurity-1, Lazer Nitroso Impurity-2, and Lazer Nitroso Impurity-3.

Component	LOD		LOQ	
	Conc.(µg/mL)	S/N	Conc.(µg/mL)	S/N
Lazer Nitroso Imp-1	0.30	3	1.00	10
Lazertinib	0.30	3	1.00	10
Lazer Nitroso Imp-2	0.30	3	1.00	10

Lazer Nitroso Imp-3	0.30	3	1.00	10
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Robustness

Robustness was investigated by deliberately varying the flow rate and mobile-phase composition around their optimized values. The nominal flow rate of 0.20 mL min⁻¹ was varied to 0.18 and 0.22 mL min⁻¹, while the nominal mobile-phase composition of 40:60 (v/v) was modified to 36:64 and 44:56 (v/v). At the lower flow rate of 0.18 mL min⁻¹, the observed %RSD was 0.87%, whereas increasing the flow rate to 0.22 mL min⁻¹ resulted in a %RSD of 0.48%. Modification of the mobile-phase composition to 36:64 and 44:56 (v/v) resulted in %RSD values of 0.54 and 0.58%, respectively. The relatively low variability observed following deliberate changes in the chromatographic parameters indicated that the analytical performance remained consistent around the optimized conditions. These findings support the robustness of the developed UPLC method.

Forced degradation and stability-indicating capability

The stability-indicating capability of the developed method was evaluated through forced degradation of Lazertinib under acidic, alkaline, oxidative, reductive, thermal, photolytic, and hydrolytic conditions. The control sample showed a total impurity level of 2.00%. Following stress treatment, differences in the chromatographic response of Lazertinib and the associated impurities were observed depending on the stress condition. Under acidic conditions, the total impurity level increased to 2.72%, accompanied by the appearance of an unknown impurity peak with an area of 58,031. Under alkaline stress, the total impurity level increased to 2.46%, with an unknown impurity peak area of 34,174. The oxidative condition produced the highest reported total impurity level of 2.82%, together with an unknown impurity peak area of 66,410.

In contrast, relatively limited changes were observed under reductive, photolytic, and hydrolytic conditions. The total impurity levels were 2.01, 2.02, and 2.00%, respectively, compared with 2.00% for the control sample. No unknown impurity peak area was reported in the corresponding table for these conditions. The thermal stress condition resulted in a total impurity level of 2.61%, with an unknown impurity peak area of 45,107. The observed increase in total impurities under acidic, alkaline, oxidative, and thermal stress conditions demonstrated greater degradation of Lazertinib under these conditions, whereas comparatively smaller changes were observed following reduction, photolytic exposure, and hydrolysis. Importantly, the chromatographic procedure enabled the monitoring of Lazertinib

together with the three specified nitroso impurities in the presence of stress-induced additional chromatographic components. The separation obtained under the optimized conditions therefore demonstrated the applicability of the procedure for stability-indicating analysis of Lazertinib. The forced-degradation results are summarized in **Table 5** & **Fig. 6**.

Table 5. Forced-degradation results of Lazertinib

Stress condition	Lazertinib peak area	Impurity -1 area	Impurity -2 area	Impurity -3 area	Unknown impurity area	Total impurities (%)
Control	15,2	90,5	121, 84	93,6	—	2.00
Acid	13,2	88,6	120, 34	92,3	58,0	2.72
Alkali	13,6	87,3	121, 17	93,1	34,1	2.46
Peroxide	13,0	89,9	118, 54	92,9	66,4	2.82
Reduction	14,9	89,2	119, 76	90,9	—	2.01
Photolytic	14,9	88,9	120, 34	91,3	—	2.02
Hydrolysis	15,1	88,7	121, 07	91,9	—	2.00
Thermal	13,3	88,5	120, 77	93,5	45,1	2.61

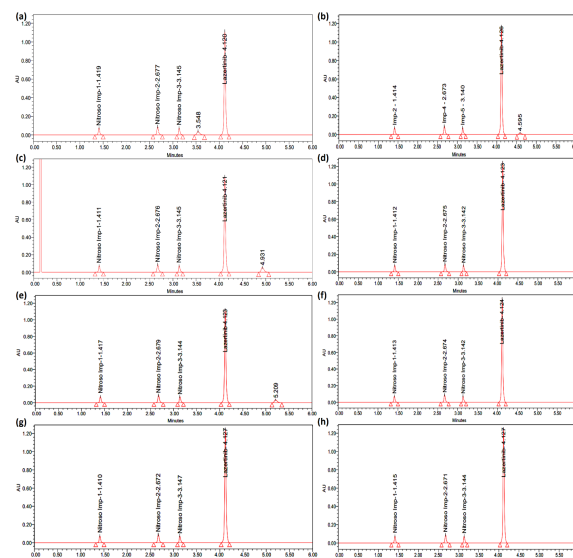


Fig. 6. Representative chromatograms obtained during forced degradation studies of Lazertinib under (a) acidic, (b) alkaline, (c) oxidative, (d) reductive, (e) photolytic, (f) hydrolytic, and (g) thermal stress conditions, together with (h) unstressed control. The chromatograms demonstrate the formation of additional degradation-related peaks under selected stress conditions while maintaining chromatographic separation of Lazertinib and the three nitroso impurities.

CONCLUSION

A rapid, reliable, and stability-indicating UPLC method was successfully developed and validated for the simultaneous determination of Lazertinib and its three specified nitroso impurities. The method provided satisfactory chromatographic separation within a short analysis time and demonstrated adequate specificity in the presence of the pharmaceutical matrix and degradation-related components. The validated method showed acceptable linearity, accuracy, precision, intermediate precision, sensitivity, and robustness, confirming its reliability for quantitative analysis. Forced degradation studies further demonstrated the suitability of the method for monitoring Lazertinib in the presence of degradation products generated under different stress conditions. Overall, the developed UPLC method provides a simple, precise, and efficient analytical procedure for the simultaneous determination of Lazertinib and its nitroso impurities and can be applied for routine quality control and stability-indicating assessment of Lazertinib pharmaceutical preparations.

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