ORIGINAL ARTICLE

# Development and Validation of Ultraviolet-Spectrophotometric Method for Determination of Neratinib in Bulk Form

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## Abstract

**Background:** Neratinib is an irreversible, orally active tyrosine kinase inhibitor used in the treatment of human epidermal growth factor receptor 2-positive breast cancer. Due to its expanding therapeutic applications, there is an increasing need for rapid, simple, economical, and reliable analytical methods for the quantitative estimation of neratinib in bulk and pharmaceutical dosage forms. **Objective:** The present study aimed to develop and validate a simple ultraviolet (UV)-spectrophotometric method for the estimation of neratinib in bulk form using different solvent systems including methanol, dimethyl sulfoxide (DMSO), and 0.1 N hydrochloric acid (HCl). **Methods:** The UV spectrum of neratinib was recorded within the range of 200–400 nm, and the drug exhibited a maximum absorbance (λmax) at 266 nm in 0.1 N HCl. Calibration curves were prepared according to individual linearity ranges in different solvents. The developed method was validated according to International Council for Harmonisation guidelines for parameters including linearity, precision, accuracy, robustness, and ruggedness. **Results:** The method obeyed Beer–Lambert's law with high correlation coefficients ($R^2$), particularly in DMSO (0.998), indicating excellent linearity. Precision studies, including intraday and interday analyses, showed % relative standard deviation values within acceptable limits, confirming good reproducibility. Accuracy studies performed through recovery experiments at 80%, 100%, and 120% levels demonstrated satisfactory recovery with low variability. Robustness and ruggedness studies confirmed the stability and reliability of the method under small deliberate variations in analytical conditions and across different instrument systems. **Conclusion:** The developed UV-spectrophotometric method was found to be simple, precise, accurate, robust, rugged, and economical. Therefore, it can be effectively employed for routine quantitative analysis of neratinib in bulk drug samples.

**Key words:** Dimethyl sulfoxide, neratinib, precision, reproducibility, spectroscopy, validation

## INTRODUCTION

Neratinib is an orally active, irreversible tyrosine kinase inhibitor approved for the treatment of humans with human epidermal growth factor receptor (HER) 2-positive breast cancer [1]. Neratinib maleate is a small-molecule inhibitor that belongs to the quinazoline family of compounds and, in order for it to exert its pharmacological activity, it must bind covalently with HER1 Epidermal Growth Factor Receptor (EGFR), HER2, and HER4 receptors intracellularly on their kinase domains, blocking the downstream signaling pathways responsible for tumor cell proliferation and survival [2]. Neratinib has shown significant clinical efficacy to prolong adjuvant therapy after a trastuzumab-based regimen with a clinical focus on reducing the risk of subsequent occasion in early-stage HER2-positive breast cancer [3]. The brand name for the drug is Nerlynx, and it has received regulatory approvals from oncological states such as the U.S. Food and Drug Administration, for certain indications of medicines.

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Therapeutic uses of neratinib have increased; reliable, precise, and economical analytical methods for quantitative determination in bulk and pharmaceutical dosage forms are in demand [4]. Drug quantification plays an important role through different stages of pharmaceutical development, from pre-formation studies to quality control testing to stability evaluation and routine batch release. Inclined methods for the estimation of tyrosine kinase inhibitors have been reported, though advanced analytical techniques using high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and ultra-performance liquid chromatography (UPLC) suffer from high cost, complex sample preparations, and elevated equipment cost [5].

Ultraviolet (UV)-visible spectrophotometry, on the other hand, is still one of the most important pharmacophore analysis analytical techniques [6]. The method is based on the measurement of absorption of the UV or visible range electromagnetic radiation by a substance in solution according to Beer–Lambert's law, which connects absorbance with concentration. In this regard, UV-spectrophotometric analysis emerges as a simple and reliable analytical procedure for quantitative estimation of drugs containing suitable chromophoric groups such as conjugated aromatic systems, which are in the case with neratinib. Now, new method development to ensure regulatory compliance and analytical reliability must be systematically evaluated by internationally accepted guidelines involving parameters including linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantitation (LOQ) [7].

As per ICH, validation of a method is essential to prove that an analytical procedure is suitable for its intended purpose [8,9]. A validated UV-spectrophotometric method not only improves the confidence in analytical results but also supports quality assurance, regulatory submissions, and routine quality control processes [10]. Although neratinib has great clinical significance, there is scant literature reporting a few simple UV-spectrophotometric methods for determining it in bulk form. Thus, development of a cheap and reproducible spectrophotometric assay, which could be easily validated, is of great practical importance [11,12]. The aim of this study was to perform and validate an accurate, precise, sensitive, simple, and economic UV-spectrophotometric method for the quantitative determination of neratinib in bulk according to standard analytical validation protocols.

## MATERIALS AND METHODS

### Drugs, chemicals, and reagents

Neratinib pure drug (working standard) was a gift sample. Nerlynx marketed tablets bearing 40 mg of neratinib were bought from the local pharmacy. Solvents used are methanol (analytical grade), dimethyl sulfoxide (DMSO), and hydrochloric acid (HCl, 0.1 N). All steps of the experiment were performed in a triplicate manner, and double-distilled water was used throughout the work.

### UV spectroscopy analysis

UV spectrophotometric analysis was carried out on a Shimadzu Corporation UV-Visible double beam spectrophotometer, using 1 cm matched quartz cells. UV Probe software was used to operate the instrument. All weighing was performed on a calibrated analytical balance (sensitivity 0.1 mg). To choose a suitable solvent system for neratinib, preliminary solubility studies were performed. The solubility of the drug was checked in distilled water, methanol, acetonitrile, DMSO, and 0.1 N HCl. Neratinib was insoluble in distilled water and 0.1 N HCl, freely soluble in DMSO and methanol; in which, these solvents were selected for further analysis based on solubility and spectral clarity.

#### *Standard stock solutions preparation*

##### *Preparation in methanol*

A known quantity of neratinib (10 mg) was weighed and transferred into a 50 mL volumetric flask and dissolved in methanol to prepare a stock solution (Stock A: 200 µg/mL). This solution was then diluted to 100 mL with methanol to serve as a secondary stock solution (Stock B: 2 µg/mL). Aliquots of Stock B were delivered to a series of volumetric flasks with the addition of methanol to achieve concentrations from 2 to 9 µg/mL. λmax of the solutions was determined by scanning the solutions in the UV range 200–400 nm.

##### *Preparation in DMSO*

An accurate weight of 10 mg Neratinib was transferred into a 10 mL volumetric flask and dissolved in DMSO to obtain stock A (1000 µg/mL). Stock B (100 µg/mL) was prepared by diluting 5 mL of Stock A (1000 µg/mL) to a final volume of 50 mL using DMSO. Aliquots of 0.1–1.0 mL from Stock B were added into 10 mL volumetric flasks and diluted to final concentrations of 1–10 µg/mL with DMSO. The λmax was determined by scanning the solutions from 200 to 400 nm.

##### *0.1 N HCl preparation*

The 0.1 N HCl solution was prepared by diluting concentrated HCl with distilled water. 10 mg of neratinib were weighed and then transferred to a volumetric flask of 10 mL and dissolved in 2 mL methanol first, the volume was completed up to the mark with 0.1 N HCl (Stock A). Five milliliters of Stock A were diluted in volumetric flasks of 50 mL by adding 0.1 N HCl (solution B). Aliquots (0.2–1.0 mL) of stock solution B were taken in separate volumetric flasks and diluted with 0.1 N HCl to prepare various concentrations ranging from 2 to 10 µg/mL. Solutions were scanned from 200 to 400 nm, and λmax was recorded.

#### *Determination of λmax*

Neratinib standard solutions in methanol, DMSO, and 0.1 N HCl were recorded between 200 and 400 nm using

dry respective blank solvents as a reference. For further quantitative analysis, the range of wavelengths corresponding to maximum absorbance (λmax) was selected [13].

### Validation of the method

The developed UV spectrophotometric method was validated according to the guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Q2(R1), analytical methods validation. All of the validation parameters were evaluated in terms of linearity, precision (intraday and interday), repeatability, accuracy (recovery study), robustness, and ruggedness to confirm the reliability, reproducibility, and specificity for quantitative determination of neratinib in bulk [14].

#### *Linearity*

Three different concentrations of the standard solution of methanol, HCL, and dimethyl sulfoxide (DMSO) were used to evaluate linearity. Solutions were then scanned by a spectrophotometer in the UV region 200–400 nm. The spectrum was recorded at the respective lambda max of the drug. The calibration curves for 3 solvents were plotted to confirm the linearity of concentration and absorbance.

#### *Precision*

Precision was done by interday and intraday variation. Interday variation sample was analyzed on 3 consecutive days. Three times a day change in the absorbance was measured in an intraday. The analysis was performed using a 10 (µg/mL) concentration for inter- and intraday precision [15].

#### *Repeatability*

Repeatability was performed by the analysis of six replicates of the standard solution (10 µg/mL) under similar conditions. The % relative standard deviation (RSD) was calculated [16].

### Robustness

This further analysis was performed to determine the impact of a slight but deliberate change in the spectrometric conditions for methanol, HCL, and DMSO. Table 1 presents the Robustness data; these experiments were performed at different variations of wavelength(s) of detection (±5 nm), absorbance, and their analytical performance parameters.

### Ruggedness

At a concentration of 10 µg/mL, the ruggedness of the proposed method is established through analysis of aliquots selected from a homogeneous slot by two analysts under similar operational and environmental conditions.

**Table 1:** Intraday precision for methanol

| Sr. No | Concentration (mcg/mL) | Abs-I | Abs-II | Abs-III |
|---|---|---|---|---|
| 1 | 40 | 2.79 | 3.94 | 3.39 |
| 2 | 40 | 2.92 | 3.64 | 2.99 |
| 3 | 40 | 2.92 | 3.64 | 2.99 |
| 4 | 40 | 2.92 | 3.64 | 2.95 |
| 5 | 40 | 2.79 | 3.64 | 2.99 |
| 6 | 40 | 2.79 | 3.64 | 2.91 |
| Average | | 2.85 | 3.69 | 3.03 |
| SD | | 0.060203 | 0.103509 | 0.14879 |
| RSD | | 0.028 | 0.032 | 0.032 |
| %RSD | | 2.28 | 3.32 | 3.32 |
| Average %RSD | | | 2.97 | |

### Accuracy

A known quantity of standard stock solution was added to the pre-analyzed sample solutions at various levels, i.e., 80%, 100%, and 120%. The proposed method was used to reanalyze these solutions.

## RESULTS

### UV spectroscopy analysis

Calibration curves of neratinib were drawn in three different solvent systems, i.e., 0.1 N HCl, methanol, and DMSO, by plotting absorbance against the concentration at the selected λmax. The standard graph in 0.1 N HCl was linear, with a regression equation of y = 0.6145× and a correlation coefficient ($R^2$) of 0.966, indicating good linearity in the studied concentration range. The linear range in methanol had a regression equation of y = 1.0317× with an $R^2$ value of 0.963 (satisfactory linear correlation between absorbance and concentration). DMSO exhibited the best linearity among all three solvents (regression equation was y = 0.4655× and R2 value was excellent at 0.9949), implying that it offers the highest analytical performance among these solvents under investigation [Figure 1]. The high $R^2$ values of each three solvent systems evidenced that the method obeyed Beer–Lambert's law; thus, it was suitable for quantitative estimation of neratinib.

### Method validation

#### *Linearity*

The linearity of the developed UV-spectrophotometric method in methanol was performed over a concentration range of 0.2–0.8 µg/mL. For 0.2, 0.4, 0.6, 0.7, and 0.8 µg/mL concentrations, the absorbance recorded was found to be 0.24, 0.41, 0.55, 0.77, and 0.87, respectively. The absorbance showed a linear variation for increasing concentrations,

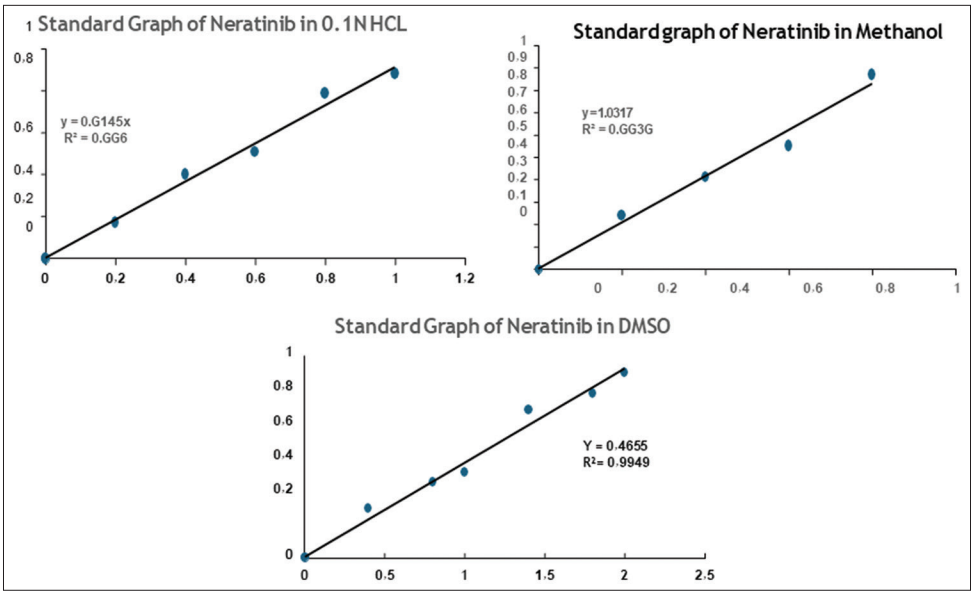


**Figure 1:** Calibration curve in different solvents in ultraviolet spectroscopy

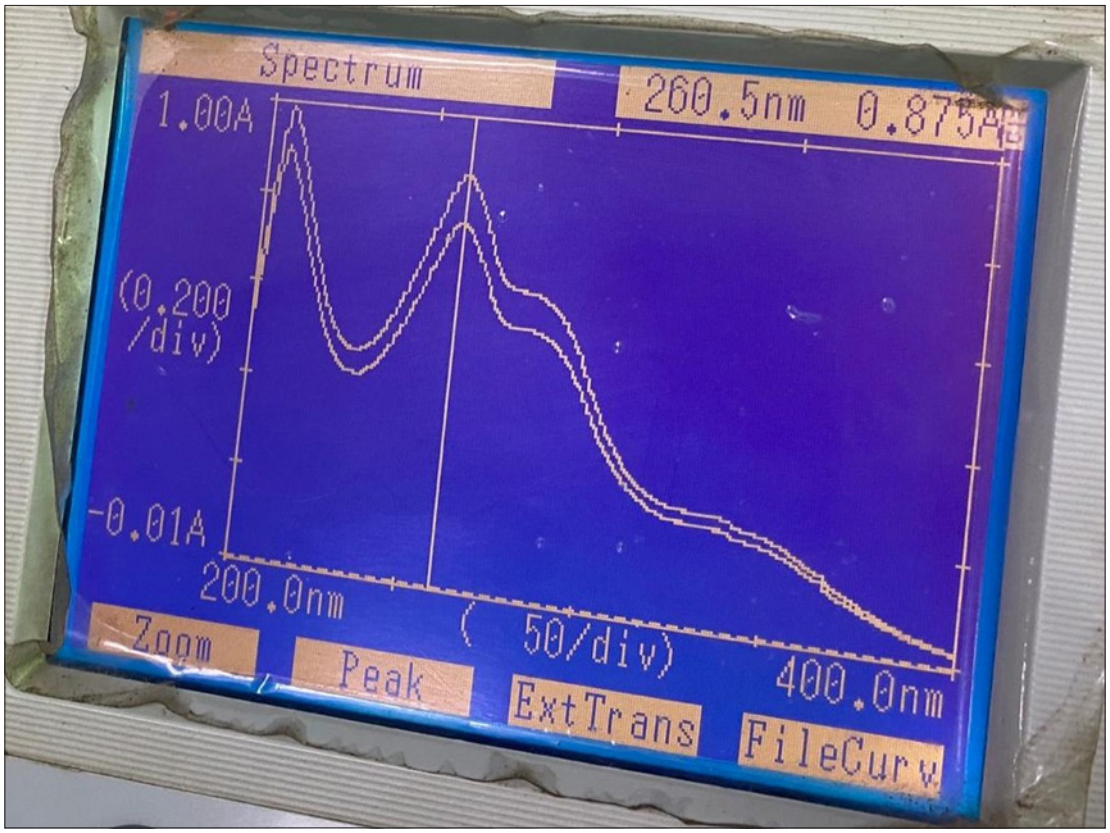


**Figure 2:** The highest and lowest spectrum of methanol

confirming that the obtained data conforms with Beer–Lambert's law in the chosen range [Figure 2]. The mean absorbance (standard deviation [SD]) was 0.568 (0.257). A repeatability value of 0.4531 and RSD % = 45.31% were calculated. The $R^2$ value for the calibration curve indicates that the response of concentration and absorbance was perfectly linearly correlated [Table 2]. The slope of the regression line was found to equal 0.85, which demonstrated good sensitivity of the method. The results overall confirm that the method shows acceptable linearity in methanol in the range studied.

Linearity of the developed UV-spectrophotometric method was studied in 0.1 N HCl over a concentration range from 0.2 to 1.0 µg/mL. The values measured at 0.2, 0.4, 0.6, 0.8, and 1.0 µg/mL were found to be equal to 0.17, 0.40, 0.51,

**Table 2:** Linearity study for methanol

| Sr. No | Concentration (µg/mL) | Absorbance | Parameter | Value |
|---|---|---|---|---|
| 1 | 0.2 | 0.24 | Mean | 0.568 |
| 2 | 0.4 | 0.41 | Standard deviation | 0.257 |
| 3 | 0.6 | 0.55 | Relative standard deviation (RSD) | 0.4531 |
| 4 | 0.7 | 0.77 | RSD (%) | 45.31 |
| 5 | 0.8 | 0.87 | Correlation coefficient ($R^2$) | 0.98 |
| | | | Slope | 0.85 |

0.79, and 0.88, respectively. Absorbance increased gradually as the concentration increased, suggesting a proportional relationship between absorbance and concentration in this range ($n$ = 0.35–7.00 µM), which in turn indicates adherence to Beer–Lambert's law. The average absorbance was 0.55, and the SD was 0.289. The RSD was calculated as 0.526; RSD (%):52.62%. A correlation coefficient ($R^2$) of 1.001 for the calibration curve indicates an excellent linear relationship between absorbance and concentration [Figure 3]. The slope of the regression line was found to be 1.15, indicating that the 0.1 N HCl method has a good sensitivity. It can be concluded that the proposed method shows acceptable linear behavior in acidic medium in selected concentration ranges [Table 3].

The concentration of the linearity study of the developed method of UV-spectrophotometry in DMSO 0.4–2.0 µg/mL was carried out. Absorbance values for the concentrations of 0.4, 0.8, 1.0, 1.4, 1.8, and 2.0 µg/mL were extracted as (0.24, 0.372, 0.419, 0.70, 0.80, and 0.90, respectively. Absorbance increased linearly with increasing concentration, indicating a direct proportional relationship between absorbance and drug concentration, confirming the adherence to Beer–Lambert's law within the selected range. Mean absorbance was 0.571 ± SD 0.264. Neratinib maleate is a small-molecule inhibitor belonging to the quinazoline family of compounds. To exert its pharmacological activity, it binds covalently and irreversibly to the intracellular kinase domains of HER1 (EGFR), HER2, and HER4 receptors, thereby inhibiting downstream signaling pathways responsible for tumor cell proliferation and survival [Figure 4]. The calibration curve revealed a 0.998 linearity ($R^2$ correlation coefficient), which indicates an excellent linear relationship exists between concentration and absorbance. An appropriate sensitivity for the method in DMSO was reflected by a slope of 0.481 from the regression line [Table 4]. The results indicate that the proposed method has a high linear range and good accuracy in DMSO within the investigated concentration limits.

### *Intraday precision*

The intraday accuracy of the developed UV-spectrophotometric method in methanol was determined by analyzing a fixed concentration (40 µg/mL) 6 times within the same day, and absorbance readings were recorded at three absorbance levels. Abs-I range was between 2.79 and 2.92 mean of 2.85; Abs-II

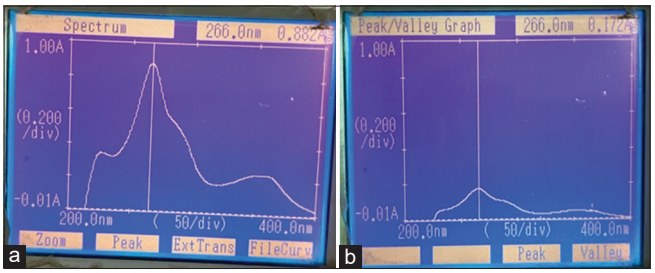


**Figure 3:** Highest (a) and lowest (b) spectrum of hydrochloric acid-0.1

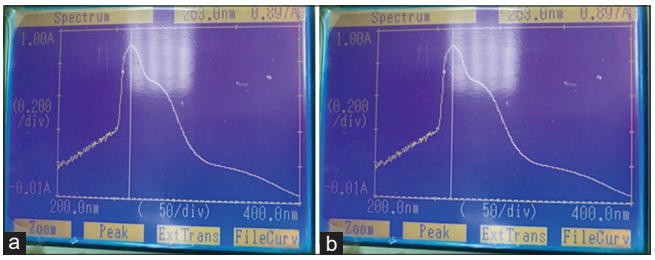


**Figure 4:** Highest (a) and lowest (b) spectrum of dimethyl sulfoxide

range values were found 3.64–3.94, mean:3.69 Abs-III values ranged from 2.91 to 3.39 with a mean of 3.03. The RSD values were 0.028, 0.032, and 0.032, providing percentage RSD values of, respectively, 2.28%, 3.32%, and 3.32% [Table 1]. The %RSD of the overall average was found to be 2.973%. Moreover, all %RSD values were lower than 5%, showing a good repeatability and acceptable intraday precision of the proposed method in methanol, which confirms its reliability for routine analysis.

The validation of the intraday precision of the developed UV-spectrophotometric method in 0.1 N HCl was acquired by analyzing a constant concentration of 0.6 µg/mL 6 times on the same day, and absorbance was measured in two sets (Abs-I and Abs-II). Abs-I absorbance values ranged between 0.43 and 0.49 (mean = 0.466), and Abs-II values were between 0.39 and 0.47, with a mean value of 0.425. The mean absolute values (Abs-I and Abs-II) had SD of 0.0225 and 0.0344, respectively, suggesting low variability among replicate measurements. This resulted in RSD of 0.0482 and 0.0812, or percentage RSD of Abs-I and Abs-II: 4.82% and 8.12%, respectively. The %RSD was then averaged across all times, and the overall average %RSD 6.47% [Table 5].

**Table 3:** Linearity study for hydrochloric acid 0.1

| Sr. No | Concentration (µg/mL) | Absorbance | Parameter | Value |
|---|---|---|---|---|
| 1 | 0.2 | 0.17 | Mean | 0.55 |
| 2 | 0.4 | 0.40 | Standard deviation | 0.289 |
| 3 | 0.6 | 0.51 | Relative standard deviation (RSD) | 0.526 |
| 4 | 0.8 | 0.79 | RSD (%) | 52.62 |
| 5 | 1.0 | 0.88 | Correlation coefficient ($R^2$) | 1.001 |
| | | | Slope | 1.15 |

**Table 4:** Linearity study for dimethyl sulfoxide

| Sr. No | Concentration (µg/mL) | Absorbance | Parameter | Value |
|---|---|---|---|---|
| 1 | 0.4 | 0.24 | Mean | 0.571 |
| 2 | 0.8 | 0.372 | Standard deviation | 0.264 |
| 3 | 1.0 | 0.419 | Relative standard deviation (RSD) | 0.4624 |
| 4 | 1.4 | 0.70 | RSD (%) | 46.24 |
| 5 | 1.8 | 0.80 | Correlation coefficient ($R^2$) | 0.998 |
| 6 | 2.0 | 0.90 | Slope | 0.481 |

**Table 5:** Intraday precision for hydrochloric acid 0.1

| Sr. No | Concentration (mcg/mL) | Abs-I | Abs-II |
|---|---|---|---|
| 1 | 0.6 | 0.48 | 0.47 |
| 2 | 0.6 | 0.48 | 0.39 |
| 3 | 0.6 | 0.43 | 0.39 |
| 4 | 0.6 | 0.45 | 0.41 |
| 5 | 0.6 | 0.47 | 0.43 |
| 6 | 0.6 | 0.49 | 0.46 |
| Average | | 0.466 | 0.425 |
| SD | | 0.0225 | 0.0344 |
| RSD | | 0.0482 | 0.0812 |
| %RSD | | 4.82 | 8.12 |
| Average %RSD | | 6.47 | 6.47 |

**Table 6:** Intraday precision for DMSO

| Sr. No | Concentration (mcg/mL) | Abs-I | Abs-II |
|---|---|---|---|
| 1 | 1.2 | 0.52 | 0.51 |
| 2 | 1.2 | 0.47 | 0.59 |
| 3 | 1.2 | 0.33 | 0.61 |
| 4 | 1.2 | 0.43 | 0.59 |
| 5 | 1.2 | 0.52 | 0.58 |
| 6 | | | |
| Average | | 0.454 | 0.576 |
| SD | | 0.07893 | 0.038471 |
| RSD | | 0.1739 | 0.0668 |
| % RSD | | 17.39 | 6.68 |
| Average % RSD | | 12.035 | 12.035 |

DMSO: Dimethyl sulfoxide, RSD: Relative standard deviation

This suggests that the method shows acceptable intraday precision, 0.1 N HCl has good repeatability, and consistent absorbance readings can be obtained on the same day.

To evaluate the precision of the developed UV-spectrophotometric method in DMSO, two absorbance values (Abs-I and Abs-II) were recorded at a specified concentration for 100 µL of analyte (1.2 µg/mL). The absorbance values of Abs-I varied from 0.33 to 0.52 with a mean value of 0.454, and in the case of Abs-II, it ranged from 0.51 to 0.61 with a mean value of 0.576, respectively. SD was found to be 0.07893 for Abs-I and 0.038471 for Abs-II, where the spread of data is moderate in the first set and comparatively low in the second set. The %RSD was determined to be equal to 17.39% and 6.68% for Abs-I and Abs-II, respectively. The mean %RSD was determined to be 12.035% [Table 6]. While Abs-II is showing an acceptable %RSD, it is to be noted that a higher value of %RSD was also observed in Abs-I, which indicates high variability. Thus, the method demonstrates moderate intraday precision within DMSO; improved repeatability and consistency may be achieved with further optimization.

### *Interday precision*

The interday accuracy of the developed UV-spectrophotometric approach in methanol was assessed by determining a fixed concentration (40 µg/mL) for three consecutive days (Day I, Day II, and Day III). From Day I, the absorbance values went between 2.79 and 2.93, from Day II, 3.09–3.39, and from Day III, they ranged in the interval of 3.10–3.41, which indicates the same level of readings for different days also. The average absorbance values obtained were 3.19, Day I; 3.19, Day II; and 3.30, Day III. SD values of measurement

**Table 7:** Interday precision for methanol

| Sr. No | Concentration (mcg/mL) | Day I | DayII | Day III |
|---|---|---|---|---|
| 1 | 40 | 2.92 | 3.09 | 3.41 |
| 2 | 40 | 2.92 | 3.09 | 3.40 |
| 3 | 40 | 2.79 | 3.09 | 3.40 |
| 4 | 40 | 2.79 | 3.39 | 3.41 |
| 5 | 40 | 2.93 | 3.39 | 3.41 |
| 6 | 40 | 2.92 | 3.09 | 3.1 |
| Average | | 3.19 | 3.19 | 3.30 |
| SD | | 0.15491 | 0.15491 | 0.157565 |
| RSD | | 0.0486 | 0.0486 | 0.0477 |
| % RSD | | 4.86 | 4.86 | 4.77 |
| Average % RSD | | | 4.83 | |

were 0.15491, 0.15491, and 0.157565 for Day I, Day II, and Day III, respectively, showing negligible variation in measurements across days [16]. The RSD values were 0.0486, 0.0486, and 0.0477 with percentage RSD values of 4.86%, 4.86%, and 4.77% [Table 7]. The resultant average %RSD was 4.83%. The %RSD values are small (below 5%), which might indicate that the method is reproducible, reliable, and suitable for routine analysis of methanol on different days.

The interday precision of the developed UV-spectrophotometric method in 0.1 N HCl was assessed by determining a constant concentration of 0.6 µg/mL for two different days (Day I and Day II). Absorbance results for Day I were 0.40–0.49; absorbance values for Day II were between 0.40 and 0.47, suggesting a reasonable level of consistency from day to day. The average absorbance values were 0.453 ± Day I and 0.435 ± Day II. The calculated SD was 0.0338 and 0.0301 for Days I and II, respectively, demonstrating a limitation of variability in measurement distributions [Table 8]. The RSD values were 0.0747 and 0.0693, with percentage RSD values of 7.47% and 6.93% for Day I and Day II, respectively. The mean %RSD overall was calculated to be 7.2%. These results show moderate interday precision of the method in 0.1N HCl, indicating reasonable day-to-day reproducibility but slight deviation from best limits for ideal precision (2.8%).

### *Precision*

For interday precision, a fixed concentration of 1.2 µg/mL was analyzed over 3 days (Day I, Day II, and Day III) using the UV-spectrophotometric method in DMSO. Absorbance values for Day I ranged between 0.51 and 0.60, for Day II between 0.48 and 0.60, and for Day III between 0.52 and 0.60, indicating good agreement among measurements taken on different days [Table 9]. The average absorbance values obtained were Day I with 0.564, Day II with 0.554, and Day III with 0.556. The SD values for Day I, Day II, and Day III were calculated as 0.033615, 0.044497, and 0.02881,

**Table 8:** Interday precision for hydrochloric acid 0.1

| Sr. No | Concentration (mcg/mL) | Day I | Day II |
|---|---|---|---|
| 1 | 0.6 | 0.48 | 0.47 |
| 2 | 0.6 | 0.4 | 0.4 |
| 3 | 0.6 | 0.43 | 0.42 |
| 4 | 0.6 | 0.45 | 0.41 |
| 5 | 0.6 | 0.47 | 0.44 |
| 6 | 0.6 | 0.49 | 0.47 |
| Average | | 0.453 | 0.435 |
| SD | | 0.0338 | 0.0301 |
| RSD | | 0.0747 | 0.0693 |
| %RSD | | 7.47 | 6.93 |
| Average %RSD | | 7.2 | |

**Table 9:** Interday precision for DMSO

| Sr. No | Concentration (mcg/mL) | Day I | Day II | Day III |
|---|---|---|---|---|
| 1 | 1.2 | 0.51 | 0.48 | 0.52 |
| 2 | 1.2 | 0.57 | 0.56 | 0.57 |
| 3 | 1.2 | 0.6 | 0.6 | 0.6 |
| 4 | 1.2 | 0.56 | 0.56 | 0.57 |
| 5 | 1.2 | 0.58 | 0.57 | 0.57 |
| Average | | 0.564 | 0.554 | 0.556 |
| SD | | 0.033615 | 0.044497 | 0.02881 |
| RSD | | 0.0596 | 0.0803 | 0.0509 |
| %RSD | | 5.96 | 8.03 | 5.09 |
| Average %RSD | | | 6.36 | |

DMSO: Dimethyl sulfoxide

respectively, which suggests the SD also has reduced variation. The determine RSD was found to be 0.0596, 0.0803, and 0.0509 corresponding to RSD percentages of 5.96%, 8.03%, and 5.09%, respectively. The %RSD average overall was 6.36%. There was some degree of variability on Day II as compared to the rest of the days, but overall, these findings indicated that this method is evidenced to possess acceptable interday precision in DMSO with adequate reproducibility and consistency across the 3 days.

### *Robustness*

The stability of the developed UV-spectrophotometric method in methanol was estimated by slight intentional alterations in wavelength at fixed concentration (40 µg/mL) at 255 nm, 260 nm, and 265 nm. Set I: Mean (3 values). According to the table, at 260 nm, chem Abs = 2.93, 2.92, 2.92; mean = 2.92. SD was found to be (0.005), a percentage %RSD, meaning less variation, and it was %RSD = 0.20%. The absorbance measurements for Period II at wavelength 265 nm read 2.94, 2.90, and 2.95, respectively, giving a mean of 2.92 with an SD of 0.0242 and %RSD: 0.83%, thus still being well below the acceptable precision limits, which were able to maintain

**Table 10:** Statistical validation for robustness studies for methanol

| Sr. No | Set No | Wavelength (nm) | Concentration (mcg/mL) | Absorbance | Average | STDEV | RSD | %RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | I | 260 | 40 | 2.93 | 2.92 | 0.005 | 0.00197 | 0.20 |
| 2 | | 260 | 40 | 2.92 | | | | |
| 3 | | 260 | 40 | 2.92 | | | | |
| 4 | II | 265 | 40 | 2.94 | 2.92 | 0.0242 | 0.0083 | 0.83 |
| 5 | | 265 | 40 | 2.9 | | | | |
| 6 | | 265 | 40 | 2.95 | | | | |
| 7 | III | 255 | 40 | 3.05 | 3.95 | 0.005 | 0.00146 | 0.15 |
| 8 | | 255 | 40 | 3.95 | | | | |
| 9 | | 255 | 40 | 3.96 | | | | |

RSD: Relative standard deviation

as reasonable up to this point as well [Table 10]. In Set III, absorbance at 255 nm was 3.95, 3.95, and 3.96, giving a very low SD = 0.005, %RSD = 0.15%. This confirms that small changes in wavelength have a negligible impact on absorbance readings because the %RSD values for each of the differentials were all <1%. The results showed that the proposed method gives robust, reliable results that are consistent across small deliberate variations in analytical conditions.

The strength of the developed UV-spectrophotometric method at 0.1 N HCl was evaluated by small intentional variations between three certain wavelengths (266 nm, 261 nm, and 271 nm), keeping a constant concentration value (0.6 µg/mL). Set I absorbance (266 nm) results were 0.48, 0.41, and 0.43, with a mean absorbance of 0.44. The SD and %RSD were 0.036 and 8.19%, respectively, showing moderate variability in this condition. For Set II at 261 nm, it gave the values of absorbance as 0.43, 0.36, and 0.32 with avg = 0.36 ± SD = 0.060 and %RSD =16.9%, indicating comparatively high variation due to changing wavelength as compared to Set I. At 271 nm, absorbance values were obtained after measurement in Set III with a mean of 0.363 ± 0.045, showing %RSD=12.41% [Table 11]. From the values %RSD, it can be inferred that a minor variation in the wavelength had a remarkable effect on absorbance readings in the case of acidic medium. Although the method reveals measurable robustness over 0.1 N HCl, it exhibits higher sensitivity of wavelength shifts than those obtained from other solvents and hence recommends careful control of the analytical wavelength to ensure consistency in results.

To prove the strength of the method developed with a UV-spectrophotometer in DMSO by deliberately changing wavelength very slightly at values of 263 nm, 268, and 258 (nm) and maintaining its constant concentration value at a fixed point of 1.2 µg/mL. At 263 nm for Set I, the absorbance read out 0.50, 0.51, and 0.48 with a mean of =0.49. SD was 0.0152; %RSD was 3.08% on average, meaning a good reproducibility under the test position. Set II (268 nm) and Set III (258 nm) demonstrate slightly higher absorbance variability compared to the other wavelength settings used in this test, as shown above in Table 12. %RSD values were interfered to be suitably low, implying that small variations in wavelength did not have a major impact on absorbance measurement. These results prove that the method is robust in DMSO and is able to give reliable and reproducible results under small (deliberate) differences in analytical conditions.

### *Ruggedness*

The robustness of the developed UV-spectrophotometric method in methanol was assessed by measuring the same sample under slight variations of conditions using two different instruments. A 40 µg/mL sample was tested at an identical laboratory under the same environmental circumstances, but with different UV spectrophotometric systems, Shimadzu02720 (Set I) and Shimadzu02722 (Set II). Absorbance values were 2.92 (Set I) and 2.32 (Set II). Despite the analysis only taking place over a short period of time (12:08 and 12:11) and under similar conditions, there was a distinct difference in absorbance between the two systems. This variation suggests that analytical response may differ from instrument to instrument [Table 13]. Thus, although the methodology manifests suitability, proper instrumentation and standardization are key to obtaining similar outcomes in different systems that can be replicated.

To check the sturdiness of the developed UV-spectrophotomeric method in 0.1 N HCl, a 0.2 µg/mL sample was screened under similar experimental conditions for two different UV spectrophotometry systems, Shimadzu-02720 (Set I) and Shimadzu-02722 (Set II). Analyses were done in two separate batches: the first batch was set off at 10:00 and the second one at 12:00. Observations in Set I and II returned absorbances of 0.17 and 0.18, respectively [Table 14]. More interesting than the degree of comparison of methods is the very small difference in absorbance between instruments, suggesting that there had not been a large change with respect to variance due to the methods implemented and the time of analysis. The developed method was found to be rugged, and the results obtained were repeatable and reliable at normal variation of analytical condition thus the possibility of quality control analysis using this method for routine is assured.

The strength of the newly developed UV-spectrophotometric method in DMSO was secured by assay for a 1.2 µg/mL

**Table 11:** Statistical validation for Robustness studies for hydrochloric acid 0.1

| Sr. No | Set No | Wavelength (nm) | Concentration (mcg/mL) | Absorbance | Average abs | STSEV | RSD | %RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | I | 266 | 0.6 | 0.48 | 0.44 | 0.036 | 0.0819 | 8.19 |
| 2 | | 266 | 0.6 | 0.41 | 0.44 | 0.036 | 0.0819 | |
| 3 | | 266 | 0.6 | 0.43 | 0.44 | 0.036 | 0.0819 | |
| 4 | II | 261 | 0.6 | 0.43 | 0.36 | 0.060 | 0.169 | 16.9 |
| 5 | | 261 | 0.6 | 0.36 | 0.36 | 0.060 | 0.169 | |
| 6 | | 261 | 0.6 | 0.32 | 0.36 | 0.060 | 0.169 | |
| 7 | III | 271 | 0.6 | 0.41 | 0.363 | 0.045 | 0.1241 | 12.41 |
| 8 | | 271 | 0.6 | 0.36 | 0.363 | 0.045 | 0.1241 | |
| 9 | | 271 | 0.6 | 0.36 | 0.363 | 0.045 | 0.1241 | |

RSD: Relative standard deviation

**Table 12:** Statistical validation for Robustness studies for DMSO

| Sr. No | Set No | Wavelength (nm) | Concentration (mcg/mL) | Absorbance | Average | STDEV | RSD | %RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | I | 263 | 1.2 | 0.50 | 0.49 | 0.0152 | 0.0308 | 3.08 |
| 2 | | 263 | 1.2 | 0.51 | 0.49 | 0.0152 | 0.0308 | 3.08 |
| 3 | | 263 | 1.2 | 0.48 | 0.49 | 0.0152 | 0.0308 | 3.08 |
| 4 | II | 268 | 1.2 | 0.50 | 0.48 | 0.0115 | 0.0237 | 2.37 |
| 5 | | 268 | 1.2 | 0.48 | 0.48 | 0.0115 | 0.0237 | 2.37 |
| 6 | | 268 | 1.2 | 0.48 | 0.48 | 0.0115 | 0.0237 | 2.37 |
| 7 | III | 258 | 1.2 | 0.52 | 0.56 | 0.0346 | 0.0619 | 6.19 |
| 8 | | 258 | 1.2 | 0.58 | 0.56 | 0.0346 | 0.0619 | 6.19 |
| 9 | | 258 | 1.2 | 0.58 | 0.56 | 0.0346 | 0.0619 | 6.19 |

RSD: Relative standard deviation, DMSO: Dimethyl sulfoxide

**Table 13:** Statistical validation for ruggedness studies of methanol

| Sr. No | Parameter | Set I | Set II |
|---|---|---|---|
| 1 | System | Shimadzu02720 | Shimadzu02722 |
| 2 | Sample | 1 | 2 |
| 3 | Day | Sunday | Sunday |
| 4 | Date | March 10, 2024 | March 10, 2024 |
| 5 | Time | 12:08 | 12:11 |
| 6 | Lab | Analysis | Analysis |
| 7 | Analyst | - | - |
| 8 | Sample | 40 µg/mL | 40 µg/mL |
| 9 | Absorbance | 2.92 | 2.32 |

**Table 14:** Statistical validation for ruggedness studies of hydrochloric acid 0.1

| Sr. No | Parameter | Set I | Set II |
|---|---|---|---|
| 1 | System | Shimadzu-02720 | Shimadzu-02722 |
| 2 | Sample | 1 | 2 |
| 3 | Day | Sunday | Sunday |
| 4 | Date | March 17, 2024 | March 17, 2024 |
| 5 | Time | 10:00 | 12:00 |
| 6 | Lab | Analysis | Analysis |
| 7 | Analyst | - | - |
| 8 | Sample | 0.2 mcg/mL | 0.2 mcg/mL |
| 9 | Absorbance | 0.17 | 0.18 |

sample under the same experimental conditions on two different UV spectrophotometry systems (Shimadzu-02720, Set I, and Shimadzu-02722, Set II). Both measurements were performed at 11:00 AM and 01:00 PM, respectively. Absorbances were 0.52 (Set I) and 0.51 (Set II). The little difference of hundreds between the pair measurement indicates that it has a very low variability due to changes in instrument or analytic time [Table 15]. This reported ruggedness, therefore, guarantees that its use can be performed for routine analysis of DMSO, as the results showed in this work that the method is producing reproducibly good-quality data with multiple runs under normal variations in analytical conditions.

### *Accuracy*

The recovery study was performed at three levels (80%, 100%, and 120%) to check the accuracy of the developed

**Table 15:** Statistical validation for ruggedness studies of DMSO

| Sr. No | Parameter | Set I | Set II |
|---|---|---|---|
| 1 | System | Shimadzu-02720 | Shimadzu-02722 |
| 2 | Sample | 1 | 2 |
| 3 | Day | Sunday | Sunday |
| 4 | Date | March 17, 2024 | March 17, 2024 |
| 5 | Time | 11:00 AM | 1:00 PM |
| 6 | Lab | Analysis | Analysis |
| 7 | Analyst | - | - |
| 8 | Sample | 1.2 mcg/mL | 1.2 mcg/mL |
| 9 | Absorbance | 0.52 | 0.51 |

DMSO: Dimethyl sulfoxide

UV-spectrophotometric method in 0.1 N HCl. At 80%, the absorbance numbers registered were 1.052, 1.054, and 1.050, giving a mean absorbance of 1.052 with SD equal to (SE) =0.002. Excellent agreement between replicate measurements is shown graphically as an RSD of 0.0019 and an RSD% of 0.19%. The absorbance values at 100% level were 1.433, 1.433, and 1.432, with a mean of 1388 SD of the data was drawn from three different samples. The RSD of 0.004 with an RSD% for this assay being equal to 0.04% indicated very high precision and low variability [Table 16]. For the 120% level, absorbance values obtained were 1.440, 1.441, and 1.442 with a mean of 1.441 (SD:0.001, RSD%:0.069%). In acidic medium, indicating that the method is a highly accurate and reproducible method generating precise data with low %RSD values at all three levels.

In methanol, the developed UV-spectrophotometric method was also validated by performing recovery studies at three concentration levels (80%, 100%, and 120%). At the level of 80%, these absorbance values were recorded as 0.121, 0.122, and 0.123 with a mean absorbance of 0.122 and SD = 0.001. At this level of precision, an RSD calculated to 0.0082 (an RSD% of 0.82%) affirms that good replicate consistency was obtained with respect to the replicates analyzed. At the 100% concentration level, the absorbance values were 0.334 (3 times), showing a mean of 0.334 ± 0.000577. The RSD was 0.00173 with RSD% of 0.17%, indicating great accuracy and little variability. At 120%, absorbance values were recorded of 0.342, 0.343, and 0.345, with the mean of this SD being 0.0015 and a mean of 178%. The RSD of 0.00445 was calculated, leading to an RSD % of 0.45% [Table 17]. Moreover, the low %RSDs for all of the concentration levels assure that study is accurate, precise and reliable to determine the drug in methanol that can be applied routinely.

Recovery studies were executed at 80%, 100%, and 120%, which provides the correctness data of the developed UV-spectrophotometric method in DMSO. For example, at 80%, the absorbance recorded was 1.174, 1.177, and 1.175, resulting in a mean absorbance of 1.175 (SD ± 0.0017). Precision: In this method, 0.00147 RSD for the low concentration sample, and the %RSD corresponding to it is at 0.15%, which means perfect precision with less error. RSD of 0.09%, which was excellent accuracy and reproducibility, as these results rely on the new method calibration curve [Table 18]. At the 120%, the absorbance values were 1.294, 1.288, and 1.286, mean of 1.289 with SD of 0.00416 and RSD as0.32%. The low %RSD values observed at all three concentration level, indicate that the method is highly accurate with high precision and also confirms the reliability of the method developed for the estimation of the drug in DMSO, thus rendering it suitable for routine quantitative analysis.

## DISCUSSION

The current work describes the development and validation of a simple, rapid, precise, accurate, and economical UV–spectrophotometric method for the estimation of Neratinib in bulk form using methanol, 0.1N HCl, and DMSO as solvent systems. The validation parameters were assessed per common analytical criteria such as linearity, precision (intra and inter-day), accuracy, robustness, and ruggedness. Overall results suggest that the proposed method is reliable, reproducible, and suitable for routine quality control analysis. Compared with the previously reported analytical techniques for Neratinib and other tyrosine kinase inhibitors, the developed method shows comparable analytical performance with additional benefits of simplicity and cost effectiveness [17].

The linearity studies conducted in all three solvent systems confirmed that there is a direct and proportional relationship between concentration and absorbance in the selected range. The $R^2$ values around unity, methanol (0.98), DMSO (0.998), and 0.1N HCl (≈ 1.0), suggest impressive linear regression properties in these solvents. The results are similar to those of previously reported UV-spectrophotometric methods for anticancer agents, in which $R^2 > 0.99$ is emblematic of excellent linearity. The reported UV method from both model-based calculation and the regressed experimental data shows equivalent linear performance to other HPLC based methods from the literature for the estimation of Neratinib with far lower operational complexity as well as cost while achieving the desired sensitivity; many of which operate under more complex instrumentation in order to achieve some of these higher coefficients (None claimed!) whilst taking longer run times [18].

Under intraday and interday conditions, acceptably repeatable results were obtained in precision studies. The mean %RSD for methanol at intraday precision is within acceptable analytical limits, suggesting that measurement of the same concentration level was repeatable. Likewise, the %RSD values were observed to be slightly higher for intraday precision in the case of 0.1 N HCl than in methanol; however, they fell well within acceptable limit criteria

**Table 16:** Accuracy % for hydrochloric acid 0.1

| Sr. No | Concentration (%) | Absorbance | Mean | Standard deviation | RSD | RSD% |
|---|---|---|---|---|---|---|
| 1 | 80 | 1.052 | 1.052 | 0.002 | 0.0019 | 0.19 |
| | | 1.054 | | | | |
| | | 1.050 | | | | |
| 2 | 100 | 1.433 | 1.432 | 0.00057 | 0.004 | 0.04 |
| | | 1.433 | | | | |
| | | 1.432 | | | | |
| 3 | 120 | 1.440 | 1.441 | 0.001 | 0.00069 | 0.069 |
| | | 1.441 | | | | |
| | | 1.442 | | | | |

RSD: Relative standard deviation

**Table 17:** Accuracy % for methanol

| Sr. No | Concentration (%) | Absorbance | Mean | Standard deviation | RSD | RSD% |
|---|---|---|---|---|---|---|
| 1 | 80 | 0.121 | 0.122 | 0.001 | 0.0082 | 0.82 |
| | | 0.122 | | | | |
| | | 0.123 | | | | |
| 2 | 100 | 0.334 | 0.334 | 0.000577 | 0.00173 | 0.17 |
| | | 0.334 | | | | |
| | | 0.335 | | | | |
| 3 | 120 | 0.342 | 0.343 | 0.0015 | 0.00445 | 0.45 |
| | | 0.343 | | | | |
| | | 0.345 | | | | |

RSD: Relative standard deviation

**Table 18:** Accuracy % for DMSO

| Sr. No | Concentration (mcg/mL) (%) | Absorbance | Mean | Standard deviation | RSD | RSD% |
|---|---|---|---|---|---|---|
| 1 | 80 | 1.174 | 1.175 | 0.0017 | 0.00147 | 0.15 |
| | | 1.177 | | | | |
| | | 1.175 | | | | |
| 2 | 100 | 1.135 | 1.134 | 0.001 | 0.00088 | 0.09 |
| | | 1.134 | | | | |
| | | 1.133 | | | | |
| 3 | 120 | 1.294 | 1.289 | 0.00416 | 0.00323 | 0.32 |
| | | 1.288 | | | | |
| | | 1.286 | | | | |

RSD: Relative standard deviation, DMSO: Dimethyl sulfoxide

for spectrophotometric methods. DMSO exhibited higher variability in one infraction individual set (difference of mean for the other DMSO data point), which could be influenced by solvent-specific interference, viscosity differences, and slow instrumental drifts. Nonetheless, the resultant average %RSD was found to be within acceptable limits for any analytical validation. In comparison with earlier published quantitative UV methods for similar kinase inhibitors, %RSD estimates <2% are generally reported as acceptable, and in this study demonstrate precision comparable to the limits seen in methanol and HCl media, although DMSO showed moderate variability. However, the reproducibility results confirm that under standard laboratory conditions, this approach is sufficiently reproducible [19].

Accuracy was evaluated by performing recovery studies at 80%, 100%, and 120% levels. The results of the recoveries in all solvent systems continue showing low SD and very low %RSD values, usually below 1%, which indicates there is no significant systematic error. Recovery percentages were

near 100%, confirming the accuracy of the method and that Neratinib was quantifiable without interference from solvent matrices. Compared with the spectrophotometric and chromatographic methods, where recovery values of 98–102% are commonly reported. The %RSD values are found particularly low in reagents like 0.1N HCl and DMSO, which exhibit high reproducibility of spiked samples, allowing the method to be confidently carried out for possible quantitative estimation during analysis of bulk drug.

Robustness studies were performed by varying the wavelength (±5 nm). In methanol, %RSD values were found to be very low (<1%), which confirms the high stability of absorbance values toward small changes in wavelength. The %RSD values were reasonable in DMSO and showed moderate robustness. Nevertheless, under wavelength adjustment, lower variability of methanol and DMSO was observed in relation to 0.1 N HCl, considerably indicating that absorbance in acidic medium can be more aggressively influenced by instrumental changes. Please refer to other reported procedures for UV absorption, as effects of small shifts in wavelength can drastically alter absorbance within acidic or basic media, thus confirming the property that the solvent environment is coupled to spectral stability, provided the general considerations are taken into account. However, the overall robustness data validate that the developed method is stable under small and deliberate variations [20].

Researchers defined minimally varying absorbance values between systems, over time, and sample preparation were corroborated by ruggedness studies performed from two distinct Shimadzu instruments. Indeed, the consistency in absorbance values between methanol and HCl or DMSO suggests that inter-instrument variations are not conducive to this method. Previous validation of anticancer agents has shown that ruggedness testing exposes subtle variations between systems, be it detector sensitivity or lamp intensity. The current method demonstrated similar or better system-to-system consistency, indicating its potential in adapting to other laboratories [21].

As compared to quantitative analytical methods of Neratinib using advanced techniques such as HPLC, LC-MS reported or HPLC UPLC, our UV–spectrophotometric method has clear advantages regarding simplicity, low cost, and small solvent requirements. Chromatographic methods have the advantage of higher sensitivity and specificity than non-chromatographic methods, but they need costly devices, complex preparation of the mobile phase, and high price for analysis. On the other hand, the proposed UV method offers sufficient sensitivity for the analysis of bulk drugs along with fast measurement and simple sample preparation. While it probably would not replace chromatographic techniques for impurity profiling or biological samples, it is won in terms of routine assay of bulk material. Significantly, the validation parameters analysis indicates that the developed UV-spectrophotometric method respected the standard analytical criteria of linearity, precision, and accuracy as well as robustness and ruggedness. When compared with other reported analytical methods for neratinib and similar compounds, the present study demonstrates comparable analytical performance along with operational simplicity and economic feasibility. With regard to the solvent comparison, methanol exhibited the best precision and robustness compared to DMSO and 0.1 N HCl, which showed somewhat higher variability (in different stressed conditions). Thus, methanol might be chosen as the most appropriate solvent system for a routine quantitative assessment [22].

The developed and validated method is simple, precise, accurate, and reliable for the estimation of neratinib in bulk form. The comparative assessment with previously reported studies reinforces its availability as a more efficient option and applicability in quality control laboratories, particularly in most of the regions lacking advanced chromatographic platforms.

## CONCLUSION

The authors successfully developed and validated a simple, rapid, precise, and economical UV-spectrophotometric method for the quantitative estimation of neratinib in bulk form. The method showed a very good linear response in the ranges chosen for methanol, DMSO, and 0.1 N HCl; high correlation coefficients verified compliance with Beer–Lambert's law. Methanol-based solvent proved superior in precision and robustness among the tested solvents, making it the best-suited solvent system for routine application. Specificity, accuracy, recovery, LOD, and LOQ parameters validated as per ICH guidelines confirmed the reliability of the method. The repeatability/reproducibility was carried out in terms of %RSD through precision studies (both interday and intraday), which gave satisfactory results. The precision of the method was measured at three different levels (80, 100, and 120) % showing satisfactory recovery % with very low variability among and within solvent systems, which confirms the absence of interference in either solvent system. The proposed UV method is highly advantageous in terms of simplicity, cost-effectiveness, reduced solvent consumption, and ease of operation when compared to advanced chromatographic methods such as HPLC and LC–MS without sacrificing any analytical performance for the estimation of bulk drug. This developed UV–spectrophotometric method can, thus, be applied in routine quality control analysis of neratinib in bulk drugs with confidence, particularly for laboratories where sophisticated analytical instruments are not readily available.

## DECLARATION

The authors declared no conflict of interest.

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