SPECTROPHOTOMETRIC ESTIMATION OF NERATINIB PHARMACEUTICALS: APPLICATIONS TO CHEMOMETRICS SUPPORTED ROBUSTNESS TESTING AND METHOD GREENNESS ASSESSMENT

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ABSTRACT

Neratinib is a novel anti-breast cancer agent approved for treating the early stages of breast cancer in women. Based on the non-availability of methods, the authors developed two UV spectrophotometric methods using water as a solvent. The drug shows a maximum absorption of 263 nm in water. Further, the measurement of the area under the curve within 259-269 nm was also performed. Method linearity from 2 to 64 µg mL^{-1} (for both methods) aptly quantified the drug from the in-house dosage form. Chemometrics-oriented designed experiments revealed the effect of method variables such as sampling interval and scanning speed on method performance and help to define control strategies. The assay results were within the acceptable range, reproducible (%RSD < 1 %), and rugged. Overall, these two UV spectrophotometric methods served the purpose of simple and rapid quality control of NRB present in tablet dosage forms.

Keywords: Design of experiments, greenness, neratinib, robustness, validation

INTRODUCTION

Neratinib (NRB) was recently approved by the USFDA as well as EMA for treating the early stages of breast carcinoma in women[1]. It is a tyrosine kinase inhibitor, and the pharmacological action is obtained at a minimal dose of 40 mg tablet orally for patients who are resistant to trastuzumab therapy[2,3]. The long-term use of NRB is yet to be scientifically established, as toxic side effects such as diarrhoea and liver toxicity are most commonly seen in patients[1]. Hence, the quality control results of such drug products should be based on simple yet reliable analytical methods that are commonly found in most laboratories. Ultraviolet (UV) – visible spectroscopy, being the most affordable and simplest analytical tool for this purpose, can be the best choice in this regard[4]. Also, the literature survey ensured the non-availability of a systematically developed and chemometrically validated UV spectrophotometric method for rapid assay of the tablets.

In the current study, the authors developed two simple UV spectrophotometric methods for the assay of NRB in tablets. Post optimization, the validation studies were performed according to ICH Q2 (R1) guideline[5]. Furthermore, considering the latest revisions intended for the Q2 (R1) guidance, the authors employed a systematic chemometric approach for ensuring method robustness for which the authors employed a central composite design[6]. These experimental designs are capable of assessing the effects of method variables on the method performance with minimal experimentation. They can optimize the experimental conditions based on the statistical study results. Finally, the method greenness was assessed using the National Environmental Method Index (NEMI) and Analytical GREEnness (AGREE) approaches[7,8]. Assessing the eco-friendly behaviour of the method ensures compliance with the principles of Green Analytical Chemistry (GAC)[9]. The results obtained from the assay for both methods were finally compared using a Student's t-test, and statistical significance was inferred.

MATERIALS AND METHODS

Chemicals

Analytical reagent grade dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich, Germany. Triple

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Table I: Summary of optical parameters and validation studies

Parameter	Methods	
	Method: I	Method: II
Detection (nm)	263	259-269
Sandell's sensitivity (µg cm^{-2} 0.001AU^{-1})	0.28	---
Molar Extinction Co-efficient(ε) (L mol cm^{-1})	1.314×10^5	---
Beer's range (µg mL^{-1})	2-64	2-64
Regression equation	Y=0.027x-0.033	Y=0.294x-0.540
Correlation coefficient (R^2)	0.999	0.999
Accuracy (% recovery, SD)		
80%	102.5, 0.86	102.8, 0.49
100% (8 µg mL^{-1})	102.9,0.28	101.52, 0.39
120%	101.52, 0.8	100.67, 0.39
Precision (% RSD)		
Repeatability	0.53	0.16
Intermediate	0.53	0.38
LOD (µg mL^{-1})	0.2	---
LOQ (µg mL^{-1})	2	---
Analysis of in-house tablets		
(Mean ± S.D.)	100.41±0.43	101.22±0.24

% recovery= Average of three determinations, Mean= Average of three determinations

distilled water was used for preparing dilutions. Standard NRB (purity > 99%) was from Weihua Pharma, Zhejiang, China. Filter papers (0.2 µ) from Sigma-Aldrich, Germany, were used to filter the sample solutions.

Instruments

UV visible spectrophotometer (Model: 1800, Shimadzu, Kyoto, Japan) with cuvettes made up of quartz having uniform dimensions (10 mm path length) were used for the study. Ultrasonicator from GT Sonic, Guangdong, China, was used for sample disruption and extraction purposes. Design-Expert® Software (Stat-Ease, Inc., Minneapolis, MN, USA) was used for performing the chemometrics-based robustness study. The instrument was controlled by UV Probe 2.1 software.

Preparation of standards and samples

A 5 mL volumetric flask containing 2.5 mL of DMSO and 5 mg of NRB was used to produce a standard stock solution of NRB, which was later diluted with water to get a concentration of 1 mg mL^{-1}. Further, these stocks were utilized to prepare 2, 4, 8, 16, 32 and 64 µg mL^{-1} solutions using water.

The in-house formulation with 40 mg of NRB was finely reduced in size, followed by an equivalent amount of tablet powder dissolved in 2.5 mL of DMSO. Post ultrasonication, the volume was made up with water and subjected to filtration. Afterwards, the filtration segregated the tablet matrix contents, and the filtrate was used to prepare sample solutions for analysis.

Table II: Chemometrics based method robustness study

Run	Sampling interval	Scanning speed	Absorbance
1	0	+1	0.188
2	-1	0	0.184
3	+1	+1	0.183
4	-1	+1	0.184
5	0	0	0.193
6	0	0	0.194
7	0	0	0.194
8	-1	-1	0.183
9	+1	-1	0.182
10	+1	0	0.183
11	0	0	0.193
12	0	-1	0.187
13	0	0	0.19
Coded Levels	**Actual Level**		
Low (-1)	0.2 nm	Slow	
Nominal (0)	0.5 nm	Medium	
High (+1)	1.0 nm	Fast	

Validation studies

The method validation studies complied with ICH Q2 (R1) requirements, emphasizing its revised approach to ascertain method reliability. Linearity was assessed over 2 - 64 µg mL^{-1} (six points) for the drug in water. Accuracy measurement relied on the method of standard addition considering the addition of standard drug at concentrations of 80, 100 and 120% of the nominal concentration (100 %) of the sample and calculating the recovery from the mixture. The nominal concentration for the accuracy study was 8 µg mL^{-1} of NRB. A single concentration level (8 µg mL^{-1}) was chosen for precision studies, and octuplicate observations were considered for calculations. Maximal recovery and lower % relative standard deviation (% RSD) < 1% were the qualifying criteria for measuring the accuracy and preciseness of the method. On the basis of a visual evaluation of the signal-to-noise ratio of the lowest quantity of analytes in both solvents, the limits of detection (LOD) and quantitation (LOQ) were established.

Table III: Obtained values for the polynomial equation

Coefficient	Obtained value
β_0	+0.192
β_1	-0.0005
β_2	+0.0005
β_3	+0.0
β_4	-0.007
β_5	-0.003
Model F-Value	12.62
ANOVA	P=0.0022
R^2	0.9001
Adequate precision	8.266

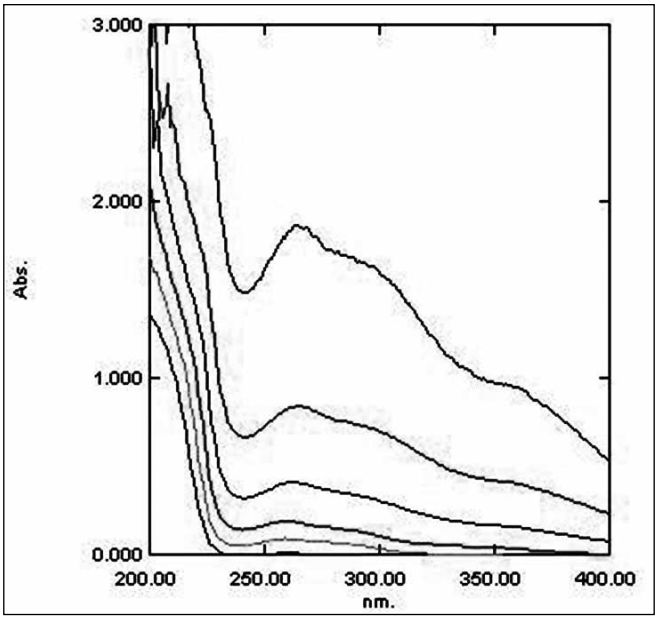

Fig. 1: Overlaid UV spectra of neratinib in calibration standards

Robustness was assessed using chemometrics-oriented designed experiments. Method variables such as sampling interval and scanning speed were investigated using a Central Composite Design (CCD). Thirteen unbiased experiments were performed, and the obtained data were studied by Analysis of Variance (ANOVA), multiple linear regression analysis (MLRA), Model F-value, adequate precision, PRESS (predicted residual error sum of squares value), perturbation chart, 2-dimensional and 3-dimensional response surface mapping followed by numerical and graphical optimization of method robustness conditions.

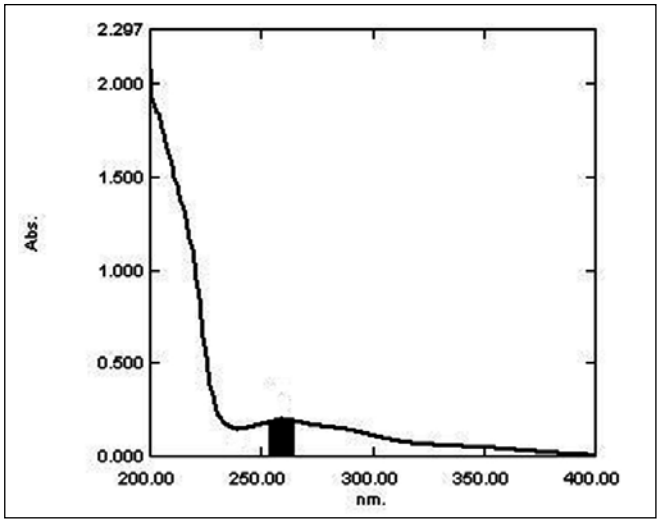

Fig. 2: UV spectrum of neratinib sample showing AUC between 259-269 nm

Method greenness assessment

The greenness of the present method was assessed by two approaches, known as the NEMI and AGREE approaches. The NEMI approach qualitatively identifies the reagents and chemicals that are hazardous (H), persistent-bioaccumulative-toxic (PBT), corrosive (C), and capable of producing waste (W), and depicts them in a pictogram. This pictogram has four quadrants for the aspects mentioned above, and a green shade indicates the green nature, whereas a white shade indicates the non-green nature of the method. In contrast, the AGREE metrics is a more comprehensive and quantitative approach that deals with all the 12 principles of GAC.

RESULTS AND DISCUSSION

Optimization of spectrophotometric method

The NRB was studied with several solvent systems and was aptly found soluble in DMSO. However, after solubilizing in DMSO, the drug was diluted further using purified water. In water, the analyte's overlaid UV spectra (Fig. 1) depicted 264 nm as the detection wavelength. Afterwards, the saved spectra were utilized to measure the AUC in the wavelength range of 259-269 nm (Fig. 2). The critical optical parameters such as molar extinction coefficient (ε) and Sandell's sensitivity were determined prior validation studies, and were satisfactory (Table I). For further investigations, the validation studies assessed the assay and method performance.

Validation summary

The linear responses for the said agent in both methods were well aligned concerning the concentrations studied. Moreover, the overall linearity was befitting, with correlation coefficients approaching unity in both cases (Fig. 3 (A-B)). Also, the statistical data suggest the aptness of the calibration curves prepared (Table I).

The accuracy and precision results for the said methods revealed maximal recovery of the analyte. At the same time, results were repeatable as well as reproducible over different days (Table I).

The visual inspection of LOD and LOQ revealed the necessary sensitivity of the methods with values suitable to linear dynamic range (Table I). Additionally, method robustness was tested, and the results indicate the optimum capability of the methods for routine application in different laboratories.

The data for 13 random experiments are given in Table II. The intended experiments as per the CCD domain were done taking into account the low, nominal, and high levels of the method variables. For absorbance at 264 nm, polynomial equations have been generated in a generalized form (Equation 1), and the corresponding coefficients are provided in Table III.

$$\text{Absorbance(Y)} = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2 \ldots (1)$$

where $\beta_0 - \beta_5 = \text{Coeficients of variables,}$

A= Sampling interval,

B= Scanning speed

Finally, we used ANOVA to statistically assess the experimental absorbance results and map the two- and three-dimensional response surfaces. Table III ANOVA findings ($P_{0.05}$) and other designed experiment attributes the suitability of the proposed design.

Fig. 4(A) shows a "dome-shaped" response surface, indicating higher absorbance values around the nominal levels of the method variables. The interpretation of the three-dimensional response surface was also strongly corroborated by a complementary set of two-dimensional contour plots (Fig. 4(B)). The perturbation plots and interaction plots (Fig. 4(C)) also display curvy factor lines, representing the response sensitivity and robust interactions between the variables under study.

The robust design space was then identified (Fig. 4(D)) using numerical and graphical optimization in the Design-Expert® software, and control limits were established for all the variables under consideration. The optimization

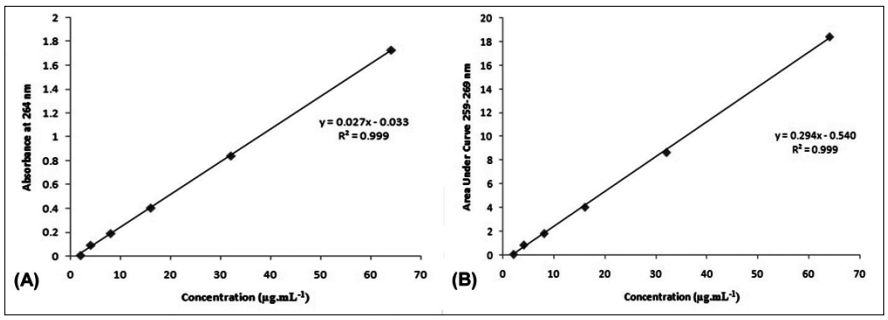

Fig. 3: Typical calibration curve of neratinib (A) Method: I and (B) Method: II

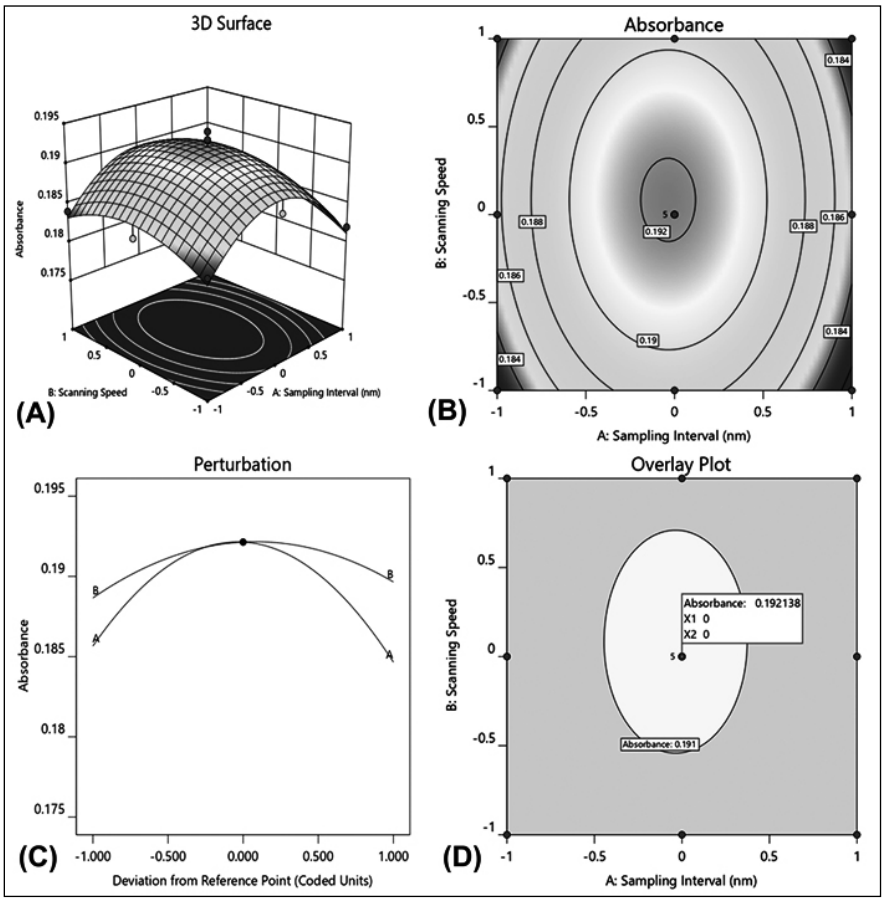

Fig. 4: Three-dimensional response surface (A), two-dimensional contour plot (B), perturbation chat (C), and robust design space (D) obtained for the DoE based robustness studies

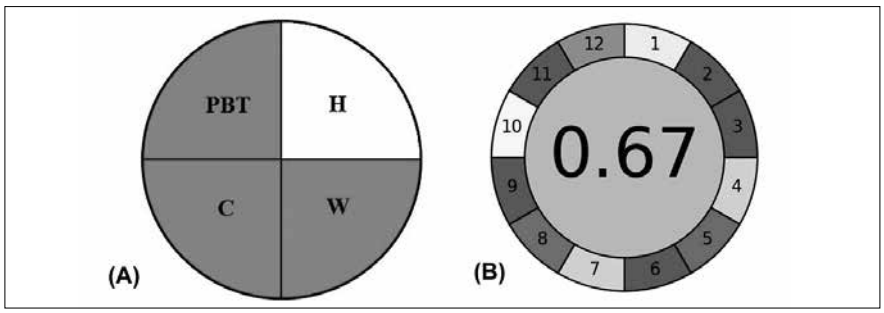

Fig. 5: NEMI (A) and AGREE (B) pictograms indicating method greenness

(desirability =1) revealed that the method would perform at its best at the nominal values of the variables under study.

Application to the assay of in-house formulations

When analyzed using the proposed methods, the authors found the in-house tablets of NRB with a comprised dose of 40 mg contain the maximal amount of the analyte (Table I). The results of a Student's *t*-test revealed a lower critical value of '*t*' at t_4 = 2.78 (P=0.05) than the calculated value (5.76), indicating a significant difference in the assay results by the proposed methods. Also, it was observed that the excipient blend used in preparing the tablets never interfered with the detection wavelength and the estimation process, indicating method selectivity and specificity.

Method greenness

Implementing the NEMI and AGREE approaches efficiently assessed the method's eco-friendly nature. Fig. 5(A) demonstrates three green quadrants in the NEMI pictogram, suggesting an excellent green aspect of the UV method. Simultaneously, the pictogram of AGREE metrics revealed only two red chambers out of the 12, and the overall greenness score was 0.67 with a matching background colour (Fig.5(B)). From the above results, it was inferred that the current UV spectrophotometric method is green and is befitting for regular use.

CONCLUSION

NRB is a new agent meant to treat breast cancer in women. Hence, for such potent agents' a reliable quality assurance and quality control are paramount. Therefore, two simple yet reliable spectrophotometric methods were developed using water as a

solvent. Afterwards, the results of optical parameters and validation studies confirmed the suitability of both methods for routine application for the assay of NRB present in tablets. Furthermore, adopting a chemometrics approach for assessing method robustness helps to establish method reliability and performance throughout the method life cycle. The method was green and environmentally caring, with satisfactory NEMI and AGREE methodology results. In conclusion, the pharmaceutical formulation of NRB may be efficiently monitored for quality using the present methodologies.

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