DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM SPECTROSCOPIC METHOD FOR ESTIMATION OF NERATINIB IN PHARMACEUTICALS: APPLICATION TO ANALYTICAL QUALITY BY DESIGN AND GREENNESS ASSESSMENT

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ABSTRACT

In this research paper, we report an eco-friendly diffuse reflectance infrared fourier transform spectroscopic (DRIFTS) method for quantifying neratinib. The systematic method development was performed as per the recent analytical quality by design concept. The critical method variables such as resolution (cm^{-1}) and the total number of scans were investigated using a central composite design to determine their influence on the measurements at 2204.64 cm^{-1}. The method was valid over a linear ($R^2 > 0.99$) range of 5-30 µg mg^{-1} of neratinib, with adequate accuracy (recovery > 100 %) and precision (RSD < 1%). Afterwards, the method greenness assessment was performed using the Analytical Greenness Metric and White Analytical Chemistry approach to establish the overall green sustainability of the current method. In a nutshell, the different functional groups of the drug were identified, and neratinib was quantified from the pharmaceutical tablets with optimum method robustness.

Keywords: Analytical quality by design, central composite design, neratinib, white analytical chemistry

INTRODUCTION

The United States Food & Drug Administration (USFDA) and European Medicine Agency (EMA) recently approved neratinib (NRB), a tyrosine kinase inhibitor, for treating women's early stages of breast cancer[1]. Chemically it is (*Z*)-but-2-enedioic acid;(*E*)-*N*-[4-[3-chloro-4-(pyridin-2-ylmethoxy)anilino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethyl amino)but-2-enamide (Fig.1)[2]. The pharmacological effect is achieved with a minimum oral intake of a 40 mg tablet for individuals who do not respond to trastuzumab treatment[2,3]. Nevertheless, the potential adverse effects of NRB, including diarrhea and liver damage, have not been definitively established by scientific research[1]. Therefore, it is strongly recommended that the quality control outcomes of these pharmaceutical drugs should be based on reliable analytical techniques that are widely employed in most laboratories[4,5].

The development of scientifically sound analytical methods can be effectively achieved by utilizing the concept of analytical quality by design (AQbD). This

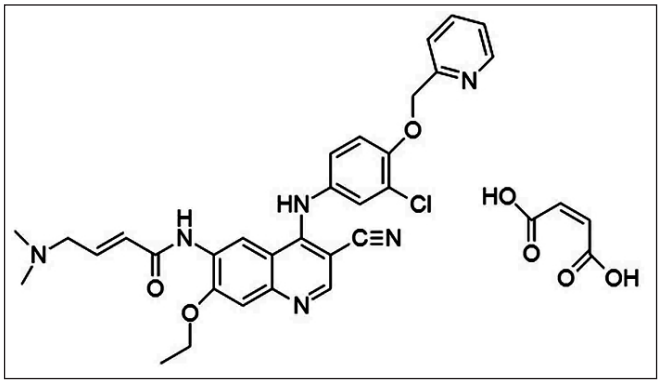

Fig. 1: Chemical structure of NRB

novel concept works on the collective principles of risk assessment and management, chemometrically supported designed experiments and statistically strengthened control strategies[6]. The pre-developmental exercises include:

- Defining an analytical target profile (ATP),
- Identifying the critical –to- quality analytical attributes (CAAs) and method variables (CMVs) from a pool of different attributes and variables, and

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Table I: Analytical target profile for DRIFTS based analysis of NRB

ATP element	Target	Rationale
(1) Analyte	(1) Neratinib (NRB)	(1) Lack of vibrational spectroscopic method for quantification.
(2) Sample Type	(2) API/Tablets	(2) As per patient's requirement.
(3) Method	(3) FTIR	(3) Apt for routine applications in powder samples.
(4) Instrumentation	(4) DRS setup with FTIR (DRIFTS)	(4) Minimum sample preparation free from use of toxic solvents.
(5) Method intent	(5) Accurate and precise assay	(5) Robust and reliable quantification of NRB.
(6) Standard preparation	(6) Accurate weighing, uniform trituration to obtain small grain size, loose sample packing and levelling	(6) Accurate dilution gives good calibration curve.
(7) Sample preparation	(7) Preliminary sample treatment, weighing, uniform trituration to obtain small grain size, loose sample packing and levelling	(7) Ensures correct dilution of the sample.
CAA	**Target**	**Rationale**
(1) Analyte peak area	(1) Maximum and robust	(1) The maximal peak area guarantees the highest level of reflectance of infra-red radiation by NRB and allows for precise estimate in samples.

- Investigating such CMVs by employing chemometrics-oriented designed experiments.

The designed experiments followed by response surface methodology (RSM) for optimizing method robustness create design space (DS) which is helpful for continuous improvement of the method throughout the method lifecycle. The DS also helps to establish the method control strategies, thereby ensuring method robustness. Furthermore, the above workflow is systematized and scientifically sound than the traditional method development exercises. Hence, counting on the advantages, such as increased scientific understanding with minimal experimental runs and greater regulatory flexibility over the method lifecycle, the authors chose to follow the AQbD approach for developing and optimizing a new method for NRB, which can efficiently quantify the analyte from pharmaceuticals. At the same time, it should simultaneously contribute to the method's eco-friendly aspect.

Green analytical chemistry (GAC) is a buzzword in analytical chemistry with lots of potential and promise for obtaining sustainable, eco-friendly methods to support a greener ecosystem[7]. Several researchers have shown a growing interest in the last half decade in developing green analytical methods to promote this concept and create awareness amongst analysts to explore greener options for routine quantification of drugs and drug products[8-11]. The GAC revolutionized analytical greenness assessment around its 12 principles, which were quite effective for the purpose[7]. The Analytical Greenness

Table II: Characteristic absorption bands obtained for NRB

Sl. No.	Wavenumber (cm^{-1})	Type of vibrations
1	3034.03, 3003.17, 2931.80, 2848.86	-C-H stretching vibrations
2	3429.43	-N-H stretching vibrations
3	2204.64	-C≡N Stretching vibrations
4	1685.79	-C=O Stretching vibrations
5	1494.83	-C=C Stretching vibrations
6	1288.45, 1267.23	-C=H Bending vibrations
7	1500-1600	-Aromatic ring

Table III: Various modes of failure investigated during FMEA study

Mode of failure	Effect	S	O	D	RPN Score
Resolution	Sharp peaks	9	9	8	648
Number of scans	Accurate peak area	8	8	7	448
Temperature	High moisture content	6	7	6	252
Grain size	Sample uniformity	5	6	4	120
Humidity	Sample integrity	5	5	4	100
Apodization mode	Improper measurement	4	5	4	80
Sample purity	Sharp peaks	5	5	3	75
Purity of KBr	Extra peaks	6	6	2	72
Range of scan	Missed characteristic peaks	4	5	3	80
Calibration	Accurate quantification	4	5	3	60
FTIR	Instrument integrity	3	4	4	60
Dilution Error	Improper peak area	3	4	2	48
Weighing Error	Improper dilution	2	3	3	24

RPN = S×O×D; S=Severity, O=Occurrence, D=Detectability

metrics approach (AGREE) is a holistic approach that adheres to the principles of GAC and generates an easy-to-interpret pictogram having a greenness score[12]. Few reported analytical applications of this greenness metrics approach are highly appreciable[13,14]. Meanwhile, recently a newer concept called white analytical chemistry (WAC) has also come up, which is more elaborate in identifying and assessing the environment-friendly methods for routine quantification[15]. WAC is an extended concept of GAC and is also considered an efficient alternative to its precedent concept. The latter is more quantitative, utilizes a Red-Green-Blue (RGB) algorithm, and provides reliable information on the method's eco-friendly nature. Under each algorithm, four principles are assessed to achieve a quantitative assessment of method whiteness. The "red" principles signify the method performance in terms of results obtained for key validation parameters. The "green" principles stress the eco-friendly nature, while the "blue" principles highlight the economy and efficiency of the analytical method. Details of these parameters are discussed elsewhere[16]. So, in view of the above

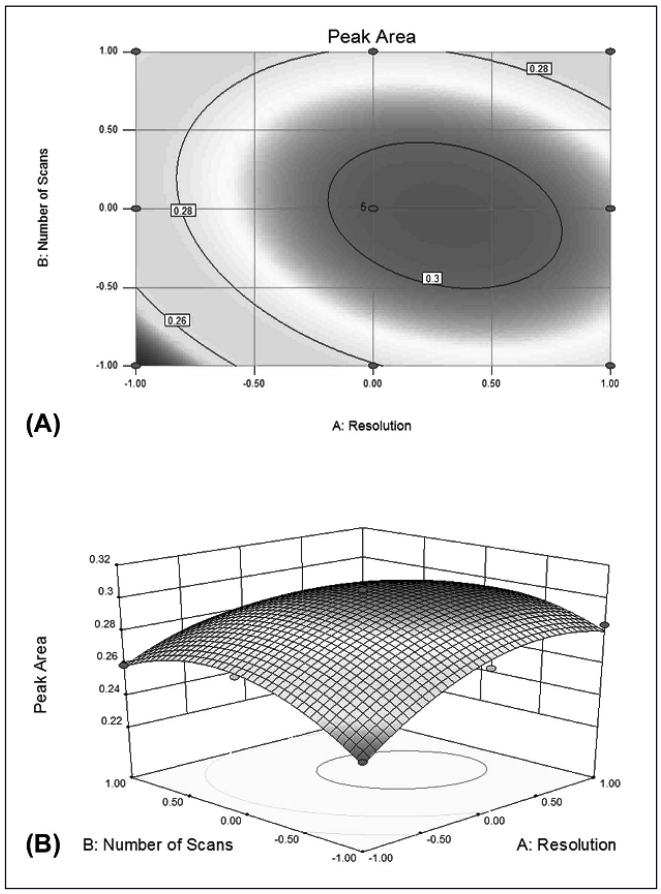

Fig. 2: Two-dimensional contour (A) and three-dimensional response surface (B) obtained during the designed experiment-based method optimization

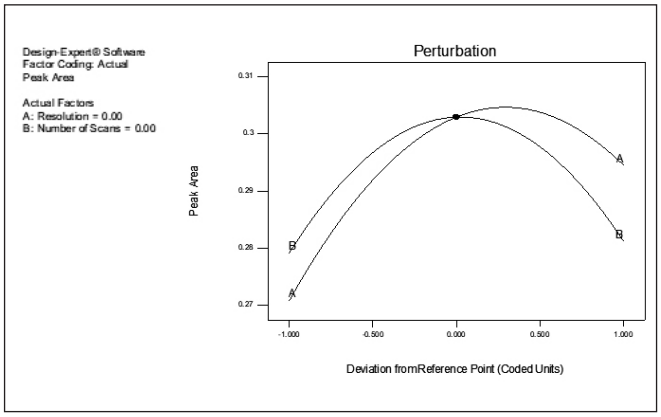

Fig. 3: Response sensitiveness for the studied CCD domain

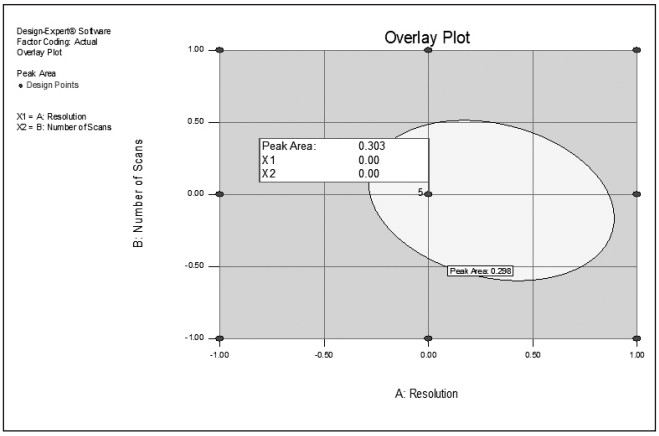

Fig. 4: DS obtained for DRIFTS analysis of NRB

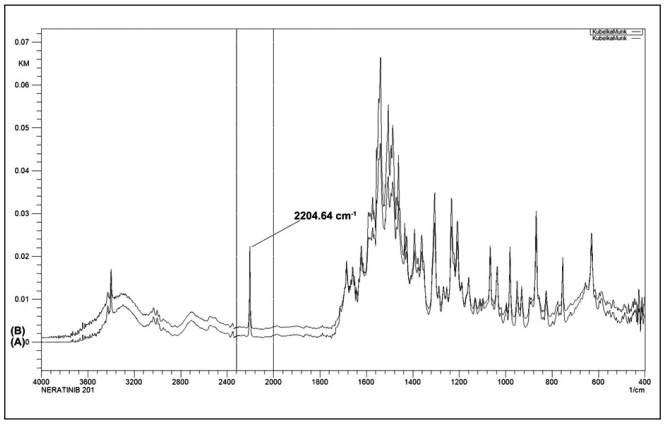

Fig. 5: Typical overlaid DRIFTS spectra of NRB standard drug (A) 5 µg mg^{-1} and (B)10 µg mg^{-1}

aspects, it becomes imperative to implement such newer approaches that promise to develop new eco-friendly analytical methods for a better environmental advent.

The literature for NRB revealed that there are only a few HPLC and LC-MS/MS methods for quantification from diverse samples[17-21]. In addition, no vibrational spectroscopic methods are reported to date for routine quantification of NRB that simultaneously identifies the functional groups present in its chemical structure. So, the authors chose to determine the analyte using the diffuse reflectance infrared fourier transfrom spectroscopy (DRIFTS) for this purpose. This technique has many advantages, like less sample preparation requirement by using KBr, fast sample cleaning, non-requirement of pressing pellets, and thereby most suitable for quantifying pharmaceuticals, etc. The theory of Kubelka-Munk (KM) is utilized to obtain the most accurate DRIFTS measurements, which consider the smallest particles of a homogeneous sample mix and loose packing of samples for analyzing pharmaceutical powders. The key benefits,

Table IV: Domain of experimentation for the current study combining robustness and optimization

Run	Resolution (cm^{-1})	Number of scans
1	-1	-1
2	-1	0
3	0	0
4	0	0
5	0	-1
6	0	1
7	-1	1
8	0	0
9	0	0
10	1	-1
11	1	0
12	1	1
13	0	0
Coded levels	Actual levels	
Low (-1)	8 cm^{-1}	40
Mid (0)	4 cm^{-1}	45
High (+1)	2 cm^{-1}	50

disadvantages, and modifications to the KM theory are discussed elsewhere[22,23].

Taking note of the enlisted facts and observations, the authors chose to develop a new DRIFTS method that is eco-friendly, reliable, robust, and flexible within ICH requirements. Method validation studies were also performed, and the current method was applied to quantify the level of NRB present in in-house tablet dosage forms. In addition, the current method's sustainability was evaluated based on the existing principles of AGREE and WAC.

MATERIALS AND METHODS

Chemicals and reagents

Standard NRB (purity > 99%) was from Weihua Pharma Co., Limited, Zhejiang, China. Spectroscopic grade KBr (Uvasol®) was from Merck, Germany. In-house tablets containing 40 mg of NRB were used for the analysis. At the same time, the placebo mixture studied contained ingredients like colloidal silicon dioxide (Parteck® SLC

500), microcrystalline cellulose (Supelco®). Crospovidone (Polyvinylpyrrolidone Powder), magnesium stearate (Parteck® LUB MST), polyethylene glycol 600, talc (Parteck® LUB MST), etc were procured from Merck, Mumbai, India.

Table V: Analytical validation data

Parameters	Results obtained
Beer's range (µg mg^{-1})	5-30
Regression equation	0.017x-0.036
Correlation coefficient (R^2)	0.999
Accuracy (% recovery, %RSD)	
80%	100.54, 0.14
100% (10 µg mg^{-1})	100.73, 0.31
120%	100.44, 0.45
Precision (%RSD)	
Repeatability	0.51
Intermediate	0.6
LOD (µg mg^{-1})	2.0
LOQ (µg mg^{-1})	5.0
Analysis of tablets (n=3)	100.23 ± 0.2
(Mean ± S.D.)	

% recovery =Average of three recoveries at each level.
Mean = Average of three determinations.

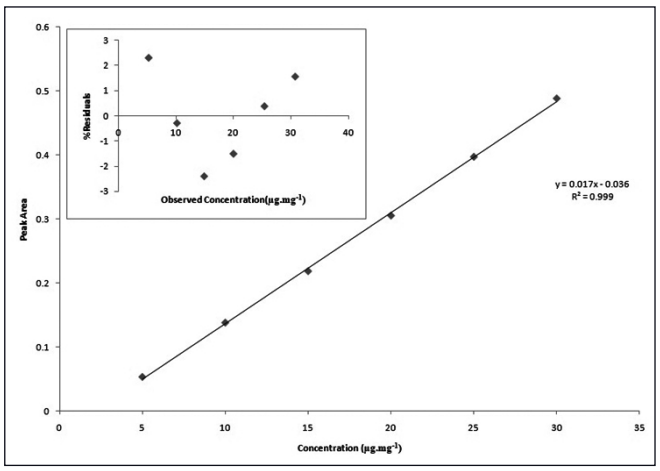

Fig. 6: Calibration curve (inset residual plot) of NRB using DRIFTS method

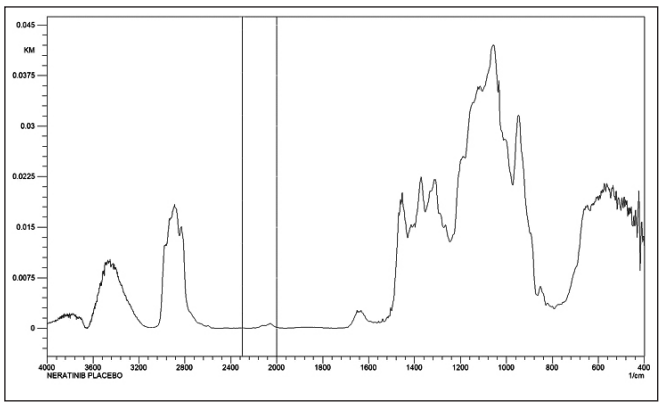

Fig. 7: DRIFTS spectra of placebo depicting non-interference at analytes measurement range

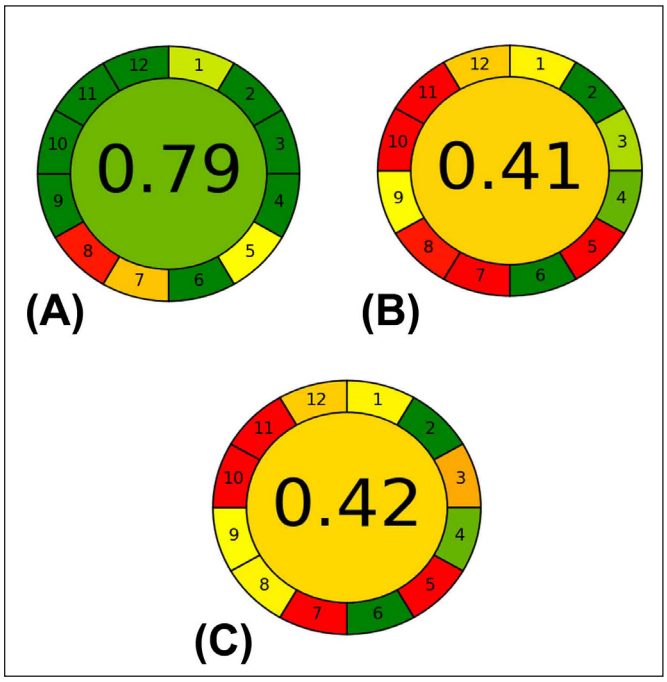

Fig. 8: AGREE metrics scores of current DRIFTS method (A) and reported LC methods (B-C)

FTIR measurement

The spectral measurements were done on a Shimadzu IR-Affinity 1 DRS8000-FTIR spectrophotometer (Shimadzu, Tokyo, Japan) controlled by IR solution software. In the Happ-Zenzell mode of apodization, each sample was scanned 45 times over 4000–400 cm^{-1} range with a resolution of 4 cm^{-1}. KBr was used for initial background correction. Before placement in the drawer type attachment, the samples were placed in a 4 mm internal diameter sample holder, surface leveled with a single swipe of blade edge, and cleaned with a fine brush to remove any spillage. The room temperature was controlled using an air-conditioner and thermometer at 27 °C while a built-in dehumidifier dehumidified the sample chamber.

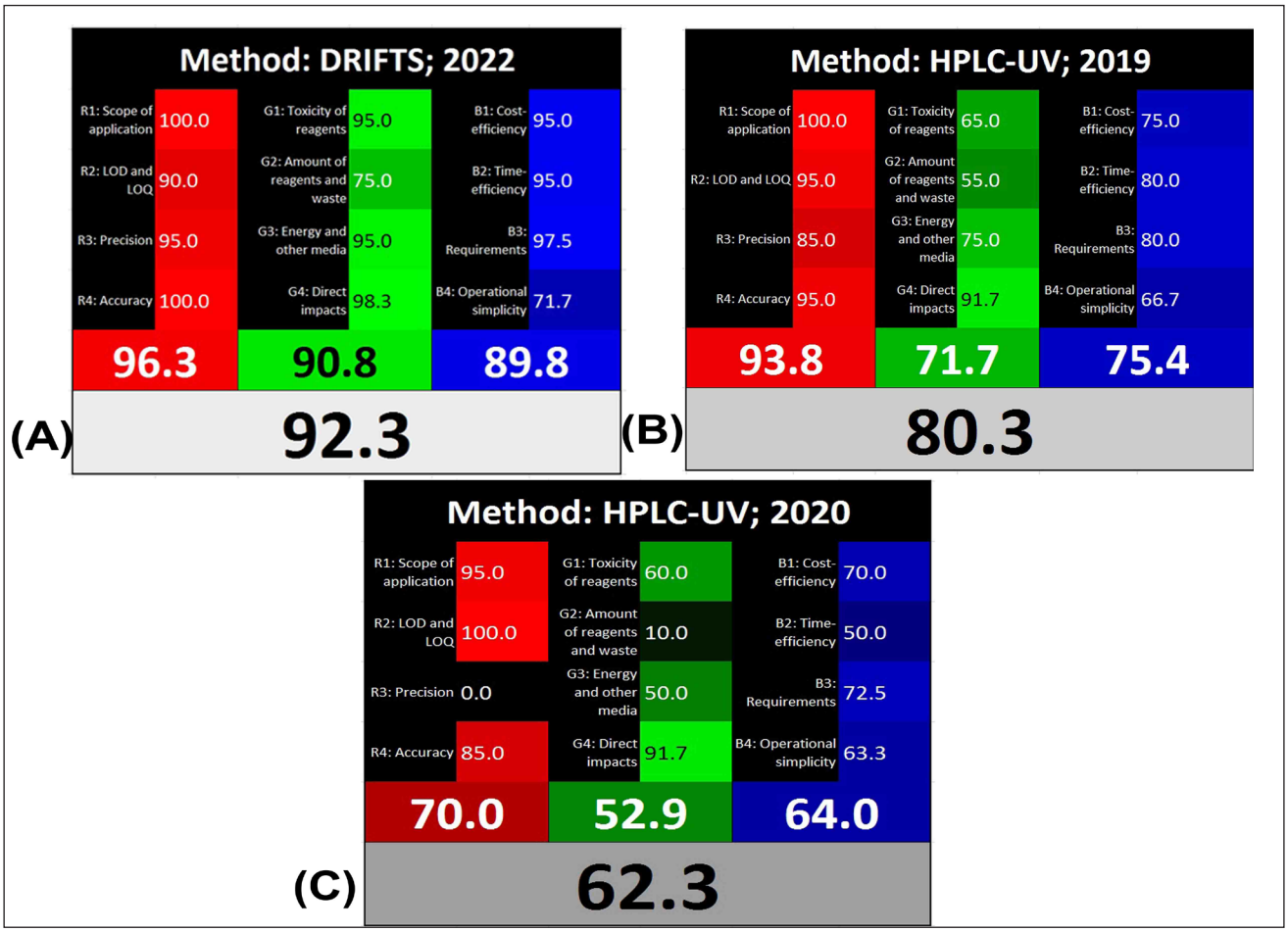

Fig. 9: WAC scores of current DRIFTS method (A) and reported LC methods (B-C)

The samples were protected from external moisture by storage inside a desiccator till final measurements. Post-acquisition, the spectra were corrected for atmospheric water (H_2O) and carbon dioxide (CO_2), and transformed into Kubelka-Munk (KM) units for precise measurements.

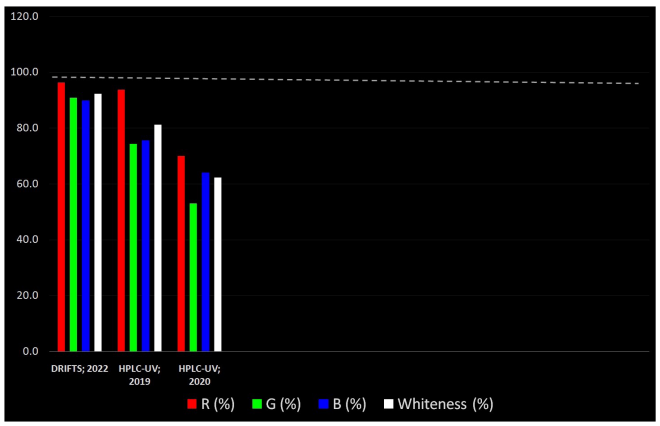

Fig. 10: Comparison of overall eco-friendly nature of the current method using WAC approach

Calibration standard preparation

Exactly 50 mg of NRB was weighed and was diluted up to 500 mg of solid KBr to produce a 100 µg mg^{-1} concentration. Six separate standard preparations with 5, 10, 15, 20, 25, and 30 mg of stock powder were accurately weighed, and a hand-held spatula-based geometric mixing technique was followed for final dilution with KBr (100 mg)[24,25]. The geometric dilution method mixes equal amounts of the analyte and diluent in a mathematical step-by-step way. This makes sure that the NRB is evenly spread out in the diluent mass, which is needed for an accurate DRIFTS analysis of powdered drugs. The resulting concentrations of NRB in KBr were 5, 10, 15, 20, 25, and 30 µg mg^{-1}. Three measurements were taken for each concentration, and the mean peak areas were in KM units, and were utilized to construct the calibration curve.

In-house tablet analysis

In alignment with our research objectives, the in-house tablets were meticulously subjected to fundamental

Table VI: Different principles of WAC and corresponding scores obtained by current DRIFTS and reported HPLC methods

Principles of WAC	Parameter	DRIFTS method (2022)	HPLC method (2019)	HPLC method (2020)
RED (Analytical Performance)	R1 : Scope for application	100	100	95
	R2 : LOD and LOQ	90	95	100
	R3 : Precision	95	85	0
	R4 : Accuracy	100	95	85
GREEN (Green Chemistry)	G1 : Toxicity of reagents	95	65	60
	G2 : Amount of reagent and waste	75	55	10
	G3 : Consumption of energy and other media	95	75	50
	G4.1 : Direct impacts	95	75	75
	G4.2 : GMO	No	No	No
	G4.3 : Animals	No	No	No
BLUE (Practical Side)	B1 : Cost efficiency	95	75	70
	B2 : Time efficiency	95	80	50
	B3 : Requirements			
	B3.1 : Sample consumption	95	75	70
	B3.2 : Other requirements	100	85	75
	B4 : Operational simplicity			
	B4.1 : MIniaturization	50	50	40
	B4.2 : Integration & automation	75	75	75
	B4.3 : Portability	90	75	75

quality control tests, such as uniformity of dosage unit, appearance, hardness, etc., to ensure the dosage form quality and suitability for the assay procedure. These tests were conducted to produce a dosage form that presents the routine challenges that are faced during the assay of a tablet. A relevant quantity of tablet powder was diluted with 500 mg of KBr after the in-house tablets were crushed to their finest form (<10 µm). Additionally, this blend was utilized to produce samples for analysis as per the measurement procedure discussed earlier.

Analytical quality by design (AQbD) approach

The ATP described the intent for method development with a suitable scientific rationale for each of them (Table I). Based on the ATP, a pool of method variables was assessed for their levels of risk on the Critical-to-Quality Analytical Attributes (CAA) using the failure modes and effect analysis (FMEA) approach. The method variables with higher Risk Priority Number (RPN) scores were identified as the Critical-to-Quality Method Variables (CMVs) and were optimized for their robust performance using chemometrics supported designed experiments. During the RPN calculation, the severity (S) score measures the potential consequences of a failure, the occurrence (O) score evaluates the possibility of the failure occurring, and the detection (D) score assesses the probability of detecting the failure. The S, O, and D scores vary from 1 to 10, with 1 being the least severe, least probable, or easiest to detect and 10 being the most severe, most likely, or hardest to detect. Finally, RPN is

determined by multiplying the scores in each category, with higher scores indicating more risk. This calculation helps to prioritize and identify method variables with high risk in an analytical method[26].

A three-level (low(-1), nominal (0), and high (+1)) two-factor response surface approach known as Central Composite Design (CCD) was utilized for the designed experiment-based robustness study and method optimization. The experimental data were subjected to quadratic mathematical modeling, where the main and interaction effects were equally considered. The results of Analysis of Variance (ANOVA) assessed the statistical validity of the selected model. In addition, numerical optimization and desirability function helped to identify the optimum experimental conditions, while graphical optimization demarcated the design space (DS).

The control strategy was defined based on the DS obtained. These strategies enabled the analyst to effectively deal with the future post-approval change control management if needed.

Validation

According to ICH Q2 (R1) specifications, the established analytical method's validation was carried out[27].

RESULTS AND DISCUSSION

Development and optimization of method

With the development of newer drug molecules, the demand for developing sound analytical methods for their quality control arises.

NRB is a new anticancer drug molecule that lacks spectroscopy methods for its routine determination. Because of this, DRIFTS is a befitting spectroscopic technique that is a simple yet economical alternative to the traditional chromatographic methods. This technique demands minimal sample preparation while simultaneously identifying the molecule's functional groups and quantifying them reliably. The sample preparation procedure of DRIFTS is an affordable alternative to the KBr pressed pellet technique used in transmission infrared spectrometric methods[28]. Also, upon literature search, the authors found that no vibrational spectroscopic techniques are reported for quantifying this novel drug molecule from its pharmaceutical dosage forms.

Given the above facts, the authors chose to apply the DRIFTS methodology for the current target drug. The pre-developmental study included scanning NRB diluted in KBr using the infra-red instrument. The room temperature of 27 °C was maintained using an air conditioner. The obtained IR spectrum shows the characteristic absorption bands corresponding to different functional portions of the molecule (Table II). NRB was found to possess a linear, distinct, and sharp absorption band at 2204.64 cm^{-1} for -C≡N stretching. However, before developing and optimizing the final set of method conditions, the robustness and performance of the method were ensured by using the AQbD concept.

The AQbD work domain was implemented, starting with identifying the target profile (Table I). After setting up the target profile, a cause-and-effect fishbone diagram was created. It revealed the sources of methodical variations that can affect the peak area at the selected infrared absorption band. Next, an FMEA assessment procedure was followed, revealing that resolution and number of scanning are the two most critical method variables (CMVs) with the highest scoring in the chart (Table III). These CMVs were further experimentally investigated by employing a response surface technique called Central Composite Design (CCD) as per the experiment domain obtained from Design-Expert® Software (Table IV). Thirteen experiments were performed under this domain, and the typical study used a quadratic model. A polynomial equation was also generated (Table V) using this model that efficiently highlighted the main and interaction effects of the CMVs as per Equation 1.

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

where $\beta_0 - \beta_5 = \text{Coeficients of variables,}$ A=Resolution (cm^{-1}) and B= Number of scans.

Afterwards, the 2-dimensional top contours and 3-dimensional response surfaces were interpreted. The "dome" shaped 3-dimensional response surface (Fig. 2(B)) was obtained, depicting maximal peak area around the nominal-levels of both the CMVs. Lower peak areas were obtained at the low-levels of CMVs which gradually increased reaching a maximal value at the nominal-levels. A similar interpretation was obtained from the top 2-dimensional contour plot (Fig. 2(A)). The perturbation chart (Fig. 3) agreed with the above plots and showed highly curved lines for both the CMVs depicting significant influence on the peak area of NRB.

The DS generated (Fig. 4) during the response surface-based optimization suggested a maximum desirability value of 1 for the final optimized conditions

of resolution of 4 cm^{-1} and a total of 45 number of scans. Matching results were obtained (Fig. 5) as predicted by the confirmatory experiments suggested by the DS. Hence, the control limits of the current analytical method were specified as 40-50 total scans and an optimum resolution of 4 cm^{-1}.

Validation report

The standard dilutions of NRB were measured thrice, and the calibration curve was prepared using peak area against concentrations (Fig. 6). The result shown in Table V vouches for the suitability of analytical linearity data. Furthermore, the validity of calibration data was supported by the % residuals *vs* observed concentration plot (inset in Fig. 6). The lowest concentrations of NRB were visually assessed to establish the detection and quantitation limits. The detection limit was determined to be 2 µg mg^{-1} with a signal-to-noise ratio (S/N) of 3, while the quantitation limit was discovered to be 5 µg mg^{-1} with a S/N of 10.

The method accuracy was calculated by considering the recovery of NRB at 80, 100, and 120 % level concentrations of 10 µg mg^{-1}. Method trueness was assured after assessing the statistical analysis results (Table V), which were found reliable.

Intraday or same-day precision and interday or different days precision was determined by analyzing eight samples (concentration 20 µg mg^{-1}) that were prepared separately. The overall results for both precision studies produced % RSD values below 1% (Table V), indicating adequate method preciseness.

Visual assessment of the DRIFTS spectra of placebo was employed to detect the possible interference of excipients at 2204.64 cm^{-1}, which are commonly used in manufacturing tablet dosage forms. The results (Fig. 7) demonstrated suitable method specificity with no interference at all at the selected region for quantification.

Assay of in-house formulation

The developed method was selective for determining the content of NRB in tablets as the % recovery was quite high (> 100%). Moreover, no interference in quantification was noticed due to the formulation excipients.

Method greenness assessment: the hyphenation of AGREE and WAC approach

The AGREE considers awarding a greenness score to each of the 12 principles of GAC. These principles include (1) sampling procedure, (2) amount of sample consumed, (3) analytical device positioning, (4) major sample preparation steps, (5) degree of automation, (6) derivatization agent used (7) amount of waste produced, (8) number of analytes analyzed, (9) most energy consuming technique, (10) reagents from renewable sources, (11) toxic reagents used and (12) operators safety. Then, according to the greenness score, the pictogram having 12 sections is assigned a specific color, where the color changes according to the non-green to green score (i.e., red to green) of the parameters. Finally, an overall green score is also awarded (0-1), considering the individual scores for all 12 principles. An overall score approaching unity is considered the greenest one. The current method shows an overall AGREE score of 0.79 (Fig. 8 (A)) compared to the two reported LC methods. In the case of the WAC approach, the 12 principles of GAC are subdivided under the RGB algorithms. These subdivisions were assigned scores according to the scoring system devised by the authors while considering some of the recent scoring systems used in this approach[29]. The results obtained from the current DRIFTS and two reported HPLC methods were compared (Table VI). The present method's analytical performance was found to be superior to the two HPLC methods in terms of method accuracy and precision (Fig. 9 (A-C)). Similarly, while assessing the method greenness parameters, the DRIFTS method was greener than the HPLC methods. Furthermore, the reagent used in the DRIFTS method has only one hazard pictogram, whereas the HPLC methods have as many as seven to eight pictograms which put serious concern regarding the method's eco-friendly nature. Also, the "blue" characteristics of the DRIFTS method were found to be superior to the reported chromatographic methods. The method whiteness of the current DRIFTS method scored an excellent 92.3 compared to the scores of the two HPLC methods (Fig. 10). Overall, the results of both the greenness assessment approaches construed the eco-friendly nature of the current analytical method.

CONCLUSION

The present research paper describes a simple DRIFTS method that is eco-friendly and flexible with the regulatory requirements for routine quantification of NRB in pharmaceuticals. The method development and its optimization are based on the principles of the AQbD concept. The CMVs resolution and the number of scanning were found to influence the peak area of NRB, which call for the adequate attention of the analyst while setting up the instrumental method parameters. In addition, control strategies were defined to obtain desired continuous method performance. Further, the principles of AGREE and WAC were implemented, and the method was found scientifically sound, eco-friendly, and efficient

for routine quantification purposes. The overall method suitability for routine quantification was optimum as the validation results matched the method intent. In a nutshell, the current integrative approach of AQbD, AGREE and WAC developed a rapid DRIFTS method for routine quality control of NRB in tablets.

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