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Original Article

ANALYTICAL QUANTIFICATION OF BOSUTINIB IN NANOCARRIER USING UV AND HPLC: METHOD DEVELOPMENT AND VALIDATION

RISHABH AGADE*, UJBAN HUSSAIN, SAGAR TRIVEDI, VEENA BELGAMWAR

Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur-440033, Maharashtra, India
***Corresponding author: Rishabh Agade; Email: rishabhagade878@gmail.com**

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ABSTRACT

Objective: Bosutinib, a potent tyrosine kinase inhibitor, holds significant promise in cancer therapy, particularly in Breast Cancer treatment. This study focuses on the analytical quantification of Bosutinib in Nanocarriers (BNCs) essential for quantification in terms of targeted delivery.

Methods: A comprehensive method development and validation process was undertaken utilizing UV-visible spectroscopy and High-Performance Liquid Chromatography (HPLC). Preformulation studies confirmed the purity and physicochemical properties of bosutinib. UV-visible spectroscopy established a calibration curve for bosutinib and BNCs, with precision, accuracy, Limits of Detection (LOD), and Limits of Quantification (LOQ) determined. HPLC analysis further validated bosutinib quantification, ensuring the robustness and reliability of the analytical method.

Results: Bosutinib and BNCs were evaluated using UV-visible spectroscopy, revealing λ max at 263 nm and 277 nm, respectively, showing a strong correlation with the regression coefficient (R^2) being 0.9969 and 0.9994, respectively. The precision (intra-day and inter-day) data shows strong reproducibility with a Percentage Relative Standard Deviation (%RSD) of less than 1.5%. Completely distinguished sharp peaks of bosutinib and BNCs were developed using HPLC under ambient settings; 3.974±0.006 and 3.083±0.004 was the Resolution Time (Rt) at which bosutinib and BNCs were discovered, respectively. The Theoretical Plate (TP) values of 5179±93 and 2598±85 and the Tailing Factor (TF) of1.00±0.002 and 1.10±0.004 were both within the predetermined bounds.

Conclusion: The developed UV and HPLC methods offer accurate and reliable quantification of bosutinib in a nanocarrier, essential for optimizing drug delivery strategies and therapeutic outcomes in cancer treatment. This analytical approach contributes to advancing pharmaceutical research in precision medicine and targeted drug delivery systems.

Keywords: Bosutinib, Lipidic nanocarriers, Cancer therapy, Drug delivery, Chromatographic analysis

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INTRODUCTION

Significant advancements have been seen in the field of medicine for combating cancer; one such advancement is the development of bosutinib, a potent tyrosine kinase inhibitor, for fighting breast cancer, being a malignancy of paramount concern in oncology. Characterized by its small molecule structure, bosutinib has emerged as a promising therapeutic agent due to its varying pharmacological properties, including potent antitumor effects and its ability to target crucial signaling pathways implicated in breast cancer progression [1]. Bosutinib is synthesized in the laboratory through a series of organic synthesis steps. Its molecular structure is characterized by a pyrimidine core fused with a quinoline ring system, aided by diverse functional groups responsible for its pharmacological activity (fig. 1). In the modern era, various approaches have been tried to optimize treatment for breast cancer, and the advent of nanotechnology in drug delivery has presented novel paths for enhancing the efficacy of bosutinib [2]. Nanocarriers loaded with bosutinib offer an appealing strategy to increase its bioavailability, improve pharmacokinetics, and facilitate targeted delivery specifically to breast tumour sites. The encapsulation of bosutinib into nanocarrier, notably lipid, holds immense potential in overcoming challenges associated with conventional bosutinib administration, such as limited solubility and inadequate tumor penetration [3, 4]. Despite these advancements, the need for the development of precise analytical techniques to evaluate Bosutinib-loaded nanocarrier (BNCs) content, release kinetics, and cellular interactions within breast cancer models remains the challenge that needs to be addressed. Robust methodologies are irrepressible to ensure the efficacy and safety of BNCs in addressing the complexities of breast cancer treatment [2]. Existing literature underscores the promising role of bosutinib and nanotechnology in breast cancer therapy; researchers have contributed to the development and validation of analytical methods for the determination of bosutinib, used in the treatment of certain types of cancer, which includes the development and validation of a UV-spectrophotometric method for the estimation of bosutinib in bulk and pharmaceutical dosage forms and establishment of a UV-spectrophotometric method for the same purpose. On the other hand, the development of a stability-indicating Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of bosutinib in bulk and pharmaceutical dosage forms focuses on its stability aspects. Another notable contribution was the stability-indicating RP-HPLC method that determine the bosutinib in the pharmaceutical formulation was made. These studies have provided valuable insights into analytical method development and validation, ensuring accurate and reliable quantification of bosutinib in various formulations [4]. The objective of this research is to advance the rapidly expanding field of nanomedicine by developing and validating an innovative analytical technique., potentially based on UV and HPLC, tailored for the quantification and characterization of bosutinib-loaded nanocarriers specifically for breast cancer applications [5].

By leveraging advanced analytical techniques, including the strategic conjugation with appropriate markers, the pivotal attributes and performance of bosutinib-loaded nanocarriers will be comprehensively elucidated, paving the way for their efficacious deployment in targeted breast cancer therapy [1].

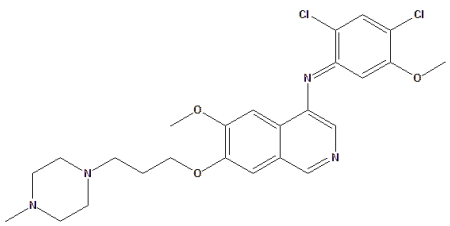

Fig. 1: Molecular structure of bosutinib

MATERIALS AND METHODS

Chemical and reagents

Bosutinib was received as a gift sample from Hetro Pharma, Ltd. India; Phospholipone 90 G was given by LIPOID®, Germany; Cholesterol and HPLC-grade Methanol were purchased from Sigma-Aldrich Corporation, Mumbai. Chloroform, Ortho Phosphoric acid, Sodium Phosphate Dibasic, and Sodium Phosphate Monobasic were procured from Merck Specialties, Pvt. Ltd., Mumbai, All the remaining chemicals and solvents were of analytical grade.

Instrumentation

The instruments used for the quantification of bosutinib and BNCs were a double Beam UV-visible spectrophotometer (JASCO V-630), having wavelength scanner range from 800 nm to 190 nm used for UV-visible spectroscopy-related studies. A silica quartz square cell cuvette with dimensions of 3 cm in length and 1 cm in route length was used for the entire experiment, having a transmittance of 50.4±0.2%. For HPLC-related studies, an Agilet (1100) with Jasco UV 2075 Plus detector coupled with autosampler, a Jasco Lc-net 11/Adc valve, a G1310A Iso pump, a C_{18} (Agilent) id (4.6 x 250 mm) column and equipped with chemstation chromatography data system for analysis was used. An analytical balance (Shimadzu, Japan), a pH meter (Systronics, Ahmedabad, India), and other instruments were used for the study.

Preformulation studies of bosutinib

The preformulation analysis and conformation of bosutinib were conducted to evaluate its purity by using various advanced methods, such as X-ray diffraction (XRD), Differential Scanning Calorimeter (DSC), Fourier Transform Infrared Spectroscopy (FTIR), followed by its organoleptic characteristics.

UV visible spectroscopy-based quantification of pure bosutinib and BNCs

Preparation of standard stock solution

The standard stock solution (1000 µg/ml) of bosutinib was prepared by precisely weighing 5 mg of the pure drug into a 5 ml volumetric flask. The drug was then dissolved with a minimum amount of methanol, and the final volume was made up to mark with methanol. This allowed for the measurement of the wavelength maximum absorption (λmax) of bosutinib [6].

Preparation of a working solution

To achieve a concentration of 10 µg/ml, the working stock solution was further diluted with methanol (1 ml to 10 ml). 1 ml of the standard stock solution was diluted to 10 ml with methanol to create the working stock solution of bosutinib (100 µg/ml).

Preparation of sample of BNCs

A sample of BNCs was prepared by measuring an accurate amount of BNCs (equivalent to 5 mg of bosutinib) and dissolving it in some amount of methanol followed by transferring the resulting solution to a 50 ml volumetric flask and making up the volume to the mark by addition of methanol to obtain a concentration of 100 µg/ml. Further, 1 ml of the prepared solution was diluted in 10 ml of methanol to obtain a solution with a final concentration of 10 µg/ml.

Selection of (λmax)

The resulting solution was scanned against methanol as a blank within the 200–400 nm wavelength range. The absorption curve displayed a distinctive absorption peak at 263 nm, and the UV-visible spectra were displayed in fig. 3 (A). Thus, (λmax) was chosen for bosutinib analysis. By further diluting the stock solution with methanol, a series of concentrations between 2 and 10 µg/ml were prepared and scanned at wavelength (263 nm) for the preparation of the calibration curve.

Validation of the analytical method

Validation of the established method was done as per ICH Q2 (R1) guideline, and several series of diluted solutions (2–10 µg/ml) were prepared and examined for linearity, precision, accuracy, LOQ and LOD [7].

Linearity and range

This method's linearity was examined at 263 nm for a range of values between 2 and 10 µg/ml (table 2). It was discovered that the bosutinib absorbance v/s concentration curve fig. 3 (B) was linear. The R^2 value was thought to be a crucial component in determining if the suggested approach was linear. The range of the suggested UV-visible approach was stated to be the range between the upper and lower concentration limits with satisfactory linearity.

Precision

The developed method was put through repeatability (intra-day) and intermediate precision (inter-day) tests to evaluate its precision. The determination of the intra-day precision involved the analysis of 10 µg/ml of bosutinib at three distinct time intervals during the day. Similar to this, the test sample concentration was used for Inter-day Precision three times over the course of three days, and the average %RSD was calculated [8].

Accuracy

Accuracy is indicated by the degree to which the results produced by the procedure are close to the true value. The accuracy of the validation was primarily assessed by recovery trials, which involved adding bosutinib to pre-analyzed sample solutions at three distinct concentrations: 8 µg/ml, 10 µg/ml, and 12 µg/ml. The percentage of recovery was then calculated [9].

LOD and LOQ

The lowest calibration concentration that can be determined with acceptable accuracy and precision is known as LOQ, while the lowest analyte concentration in a sample that an analytical method can consistently distinguish from background levels is known as LOD. Using the formula given in Eqn (1), the calibration curve's slope and the Standard Deviation (SD) of the response served as the basis for both LOD and LOQ in this procedure [8, 9].

$$\text{LOD} = 3.3\frac{S}{M};\ \text{LOQ} = 10\frac{S}{M} \text{..... Eq. (1)}$$

Where M is the calibration curve's slope and S is the sample's absorbance standard deviation.

HPLC-based quantification of pure bosutinib and BNCs

Chromatographic conditions

HPLC of bosutinib and BNCs was carried out using a C_{18}(Agilent) column and Methanol and 0.1 % Ortho Phosphoric Acid (pH 2.7) as mobile phase

Preparation of samples

Samples of Pure bosutinib and BNCs were prepared using varying proportions of Methanol and 0.1 % Ortho Phosphoric Acid (pH 2.7) using HPLC, isocratic mode at a constant flow rate of 0.7 ml/min; the estimation was carried out by maintaining the column temperature 25 ℃, the sample injection volume being 20 µl** for 10 min, and the detection was done at 250 nm (table 1).

Table 1: Chromatographic conditions for HPLC estimation of bosutinib and BNCs

S. No.	Sample	Column	Mobile phase	Flow rate	Detector
1	Bosutinib	C_{18}(Agilent)	Methanol: 0.1 % Ortho Phosphoric Acid (pH 2.7) (80:20)	0.7 ml/min	Jasco UV 2075 Plus detector
2	BNCs	C_{18}(Agilent)	Methanol: 0.1 % Ortho Phosphoric Acid (pH 2.7) (80:20)	0.7 ml/min	Jasco UV 2075 Plus detector

Preparation of pH buffer 7.4 solution

Accurately weighed sodium phosphate dibasic (Na_2HPO_4) (142.04 g), was dissolved in 800 ml of distilled water. In another beaker, 19.06 g of sodium phosphate monobasic (NaH_2PO_4) was dissolved in 200 ml of distilled water. Stirring constantly, the sodium phosphate monobasic solution was gradually added to the sodium phosphate dibasic solution, pH meter was used to measure the solution's pH after each addition. The sodium phosphate monobasic solution was added until the pH reached 7.4. Once the pH reached the desired level the volume was brought to 1000 ml by the addition of distilled water. For even mixing, the mixture was given a good stir. Finally, the prepared buffer solution was kept at room temperature in a sterile, firmly sealed container [10].

Preparation of bosutinib

Bosutinib in the mobile phase, Methanol: 0.1 % Ortho Phosphoric Acid (pH 2.7) (80:20) was used as standard. The mixture was filtered using a syringe-driven PVDF hydrophilic membrane filter (Hi-Media) having a pore diameter of 0.10 µ. Moreover, samples were injected using a 20 µl** HPLC syringe (Hamilton syringe) at room temperature (25ºC) and flow rate (0.7 ml/min). After a 10-minute run, the samples were detected at 250 nm, and a calibration curve was plotted. The estimation of BNCs is described further.

Method validation

The validation of the methods carried out by Linearity, precision, accuracy, repeatability, the LOD and LOQ of the HPLC approach for estimating bosutinib in the mobile phase was validated as per ICH guidelines Q2 (R1) [7].

The linearity of the proposed approach was assessed in the 2–10 µg/ml range using a linear regression model and the least squares method. Plotting peak area vs analyte concentration of bosutinib in Methanol: 0.1 % Ortho Phosphoric Acid (pH 2.7) (80:20) at five different standard solutions (2, 4, 6, 8, 10 µg/ml) used for the computation of the regression line [11].

To identify the precision and accuracy, repeatability and intermediate precision were used. Intraday variation was used to assess the repeatability of the developed process, which involved three trials with three different concentrations at three different times of the day. By using the interday variation technique, involving the estimation of three different concentrations on three separate days, intermediate precision was achieved simultaneously. The developed method's specified range was met by the concentrations of 2 µg/ml (low), 6 µg/ml (medium), and 10 µg/ml (high). SD and %RSD were applied to the data, which were utilized as criteria to determine intraday and interday precision [11].

By utilizing bosutinib in duplicates of three at a concentration of 10 µg/ml in the specified mobile phase, the applicability of the system was assessed. The factors taken into consideration for determining the applicability of the system were the TF, Height Equal to a Theoretical Plate (HETP), and TP.

Little adjustments were made to crucial analytical parameters, such as the ratio of mobile phase, Methanol: 0.1 % Ortho Phosphoric Acid (pH 2.7) (80:20) to increase the method's robustness. Conversely, temperature variations were observed to be 25±1 °C, and the mobile phase flow rate was adjusted to either 0.8 ml/min or 0.9 ml/min. The test solutions were concentrated at 2 µg/ml (low), 6 µg/ml (medium), and 10 µg/ml (high). RSD and the % recovery were used to evaluate the data collected to observe changes in these parameters affecting the results.

According to Eqn. (2) and (3), the calibration curve slope (S) and response (σ) measured from the minimal SD were used to compute the LOD and LOQ were later confirmed.

$$LOD = \sigma/S \times 3.3 \text{ .... Eqn. (2)}$$

$$LOQ = \sigma/S \times 10 \text{.... Eqn. (3)}$$

Statistical analysis

At least three trials were conducted for each experiment. The information was displayed as mean±SD. One-way Analysis of Variance (ANOVA) was used to compare all of the data. Various methods along with a corresponding Standardised test, Newman-Keul's test. The significant difference was established at $p<0.05$.

RESULTS AND DISCUSSION

Preformulation studies of bosutinib

An analysis of organoleptic characteristics revealed that bosutinib was odourless, white in colour, and an amorphous powder. Using a digital melting point apparatus (Electronics India 935, India), the melting point was observed to be within a range of 137–141 °C, which corresponded with the reported reference [12]. The melting point was also confirmed by DSC (Mettler-Toledo, Japan). The range of determination was 20 to 250 °C. At 147.1ºC, the DSC thermogram (fig. 2A) displayed a strong peak and end set value. Indicating crystalline nature [13]. XRD Diffractograms of bosutinib showed characteristics of intense peaks represented in fig. 2B, which confirmed the crystalline nature of bosutinib. The FTIR spectra (fig. 2C) from Shimadzu, Japan, display the appropriate band at 765, 989, 1120, 1674.15, and 2249 cm−1. The C-O stretching took place as a broad peak between 1050 and 1250 cm−1 apart from the typical amine bands, amine peak was seen at 1650–1500 cm-1.

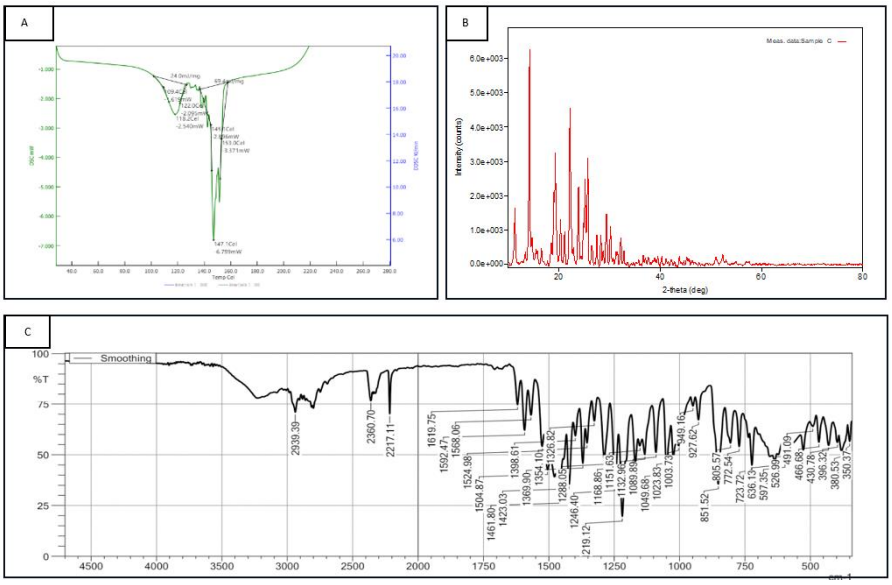

Fig. 2: Preformulation study of Bosutinib (A) DSC, (B) XRD, (C) FTIR

UV visible spectroscopy based quantification of pure bosutinib and BNCs

It was observed that bosutinib was soluble in methanol. As indicated in (fig. 3 A), the absorption maximum (λmax) was determined to be 263 nm. With a correlation value (R^2) = 0.9969 and high linearity within the concentration range of 5–25µg/ml (table 2), the regression equation of the curve was determined to be y = 0.0143x – 0.0456 at 263 nm. The absorption maximum (λmax) for BNCs was determined to be 277 nm as indicated in (fig. 4 A), having a high linearity in the concentration range of 5–25 µg/ml (table 2) with a correlation value (R^2) = 0.9994, the regression equation of the curve was determined to be y = 0.0177x+0.0329 at 277 nm (fig. 4 B). The precision (intra-day and inter-day) data shows strong reproducibility with a % RSD less than 1.5% in (table 3), indicating that the approach is precise. It was discovered that the mean recovery value (table 4, 5) at various doses was greater than 95%, the procedure is accurate. Bosutinib and BNCs LOD and LOQ were discovered to be 0.018µg/ml and 0.0482µg/ml at 263 nm, respectively, and were reported in table 6 [6, 8].

Table 2: Data for calibration curve of bosutinib at 263 nm and BNCs at 277 nm

S. No.	Concentration (µg/ml)	Absorbance of bosutinib	Absorbance of BNCs
1	5	0.113±0.02	0.121±0.08
2	10	0.191±0.11	0.214±0.21
3	15	0.261±0.09	0.294±0.15
4	20	0.341±0.22	0.385±0.3
5	25	0.396± 0.35	0.478± 0.24

All the values are expressed as mean±SD, n=3

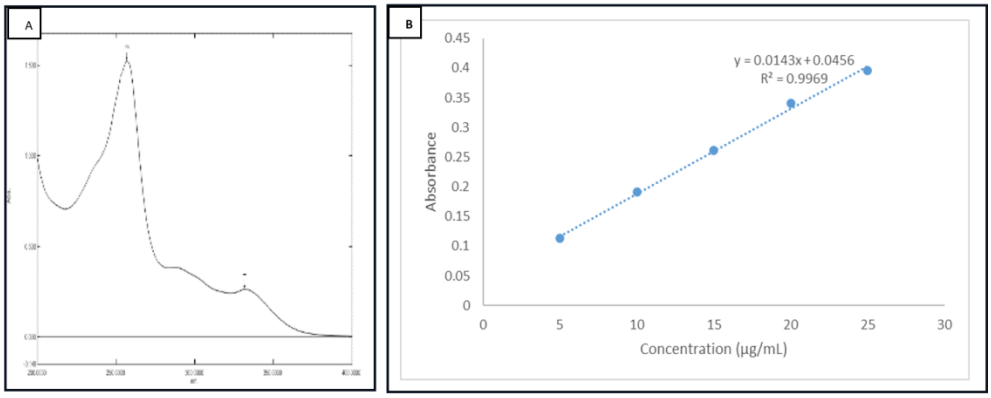

Fig. 3: (A) λmax of bosutinib, (B) Calibration curve of bosutinib at 263 nm

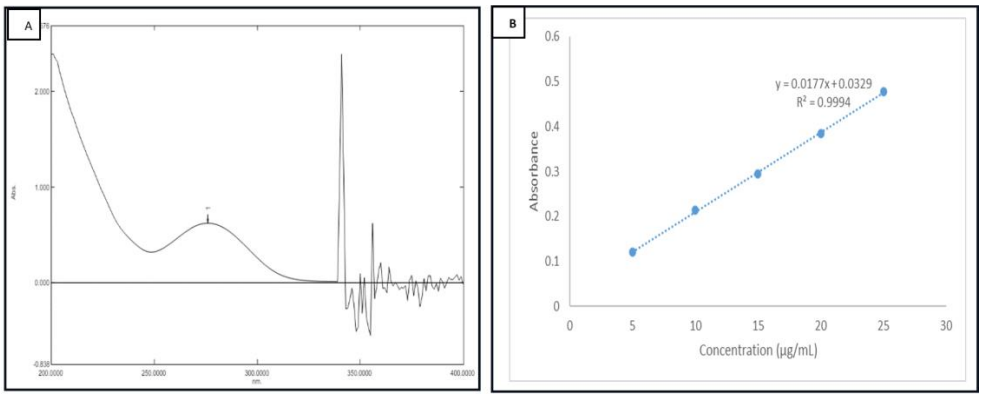

Fig. 4: (A) λmax of BNCs, (B) Calibration curve of BNCs at 277 nm

Table 3: Results of intra-and inter-day precision

Concentration (µg/ml)	Intra Day				Inter Day			
	Bosutinib		BNCs		Bosutinib		BNCs	
	Absorbance (nm)±SD	%RSD	Absorbance (nm)±SD	%RSD	Absorbance (nm)±SD	%RSD	Absorbance (nm)±SD	%RSD
25	0.396±0.002	0.0975	0.478±0.01	0.099	0.387±0.009	0.1191	0.477±0.062	0.0911
25	0.391±0.007	0.0914	0.482±0.02	0.0927	0.397±0.024	0.0991	0.487±0.057	0.0905
25	0.389±0.011	0.0951	0.480±0.061	0.0979	0.380±0.031	0.1010	0.479±0.091	0.0973

All the values are expressed as mean±SD, n=3

Table 4: Recovery study of bosutinib

λmax (nm)	Level of recovery (%)	Amount spiked recovery (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean recovery
	140	14	134.05	95.75	
263	160	16	156.33	97.71	96.71
	180	18	174.04	96.69	

Table 5: Recovery Study of BNCs

λmax (nm)	Level of recovery (%)	Amount spiked recovery (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean recovery
	150	15	13.81	92.11	
277	180	18	171.9	95.51	94.93
	200	20	19.43	97.19	

Table 6: Validation parameters

Validation parameters	Results	
	Bosutinib	BNCs
λmax	263 nm	277 nm
Beer's law range (µg/ml)	5-25µg/ml	5-25 µg/ml
Correlation Coefficient (R^2)	0.9969	0.9994
Slope (m)	0.0143	0.0177
Intercept (c)	0.0456	0.0329
Accuracy	95.09	96.79
Precision (%RSD)	0.312	0.219
Intra-day	0.09466	0.1064
Inter-day	0.09651	0.09296
LOD (µg/ml)	0.018	0.031
LOQ (µg/ml)	0.0482	0.05571

High-performance liquid chromatography

To estimate bosutinib in formulated liposomes, a validated HPLC method was developed. The established method helped evaluate the *in vitro* drug release and stability profile in addition to enabling the determination of the total encapsulation of bosutinib in liposomes [14]. The HPLC approach is superior to UV spectrophotometric assessment because it allows for approximation even at lower concentrations. In addition, this technique's simple actions make it easier to detect even at very small concentrations.

Depending on the solubility of bosutinib, different solvents such as ethanol, methanol, and 2-propanolol were tried, and their proportions were adjusted with Ortho Phosphoric Acid (pH 2.7). This was the first step in the isocratic chromatographic quantification of bosutinib. Numerous trial runs were conducted with different combinations of methanol and Ortho Phosphoric Acid (pH 2.7), and the chromatograms produced showed uneven peaks and tailing factors less than 3. Simultaneously, the tailing factor was less than 2.5 and the ratio Methanol: Ortho Phosphoric Acid (pH 2.7) provided comparatively acceptable resolution. The best separation of bosutinib was obtained using Methanol: Ortho Phosphoric Acid (pH 2.7) in a ratio of 80:20, with chromatographic parameters including a column temperature of 25 °C, an injection volume of 20 µl, a flow rate of 0.7 ml/min, and a detection wavelength of 250 nm [11]. Under ambient settings, 3.974±0.006 was the Rt at which bosutinib was discovered (fig. 5A). TP value of 5179±93 and the TF of 1.00±0.002 were both within the predetermined bounds (table 7). By maintaining the other chromatographic conditions as previously described, the primary goal of analytical profiling of bosutinib and bosutinib Loaded Nanocarrier in pH buffer 7.4 was achieved [15]. The solubility of BNCs was checked by using different solvents such as ethanol, methanol, isopropyl alcohol, and other polar solvents, BNCs were found to be soluble in methanol and its proportion was adjusted with Ortho Phosphoric Acid (pH 2.7), isocratic mode of HPLC was used for quantification of nanocarrier which showed uneven peaks and tailing factors less than 2.5. Simultaneously, the tailing factor was less than 2 and the ratio Methanol: Ortho Phosphoric Acid (pH 2.7) provided comparatively acceptable resolution. The best separation of BST-NCs was obtained using Methanol: Ortho Phosphoric Acid (pH 2.7) in a ratio of 80:20, with chromatographic parameters including a column temperature of 25 °C, an injection volume of 20 µl**, a flow rate of 0.7 ml/min, and a detection wavelength of 250 nm [16]. Under ambient settings, 3.083±0.004 was the Rt at which bosutinib was discovered (fig. 5B). The TP value of 2598±85 and the TF of 1.10±0.004 were both within the predetermined bounds (table 7).

Method validation

The calibration curve was used to verify the linearity of the devised analytical method by examining the relationship between the response and the concentration of bosutinib in the sample. A calibration curve was built (n = 3) for the bosutinib in the mobile phase Methanol: Ortho Phosphoric Acid (pH 2.7) in a ratio of 80:20 for five concentrations ranging from 2 to 10 µg/ml. The regression equations for the Methanol: Ortho Phosphoric Acid (pH 2.7) in a ratio of 80:20, respectively, were found to be y = 0.0179x-0.015; correlation coefficient (R^2) = 0.9995 and it was determined from the equations and quality of curve that the results were significant and linear, with a rise in response observed as the concentration of bosutinib in the sample increased.

The study examined the repeatability and intermediate precision of bosutinib at three distinct doses. In the mobile phase, the concentrations were 2 µg/ml (low), 6 µg/ml (medium), and 10 µg/ml (high) (table 8). The analysis was conducted on the same day (repeatability) and over three separate days (intermediate precision). The maximum %RSD values (table 9) were less than 2, indicating that the developed approach had higher precision [4, 17, 18].

Table 7: System suitability parameters

Parameter	Standard bosutinib		BNCs	
	Mean±SD	%RSD	mean ±SD	%RSD
Rt	3.974±0.006	0.152	3.083±0.004	0.13
TF	1.00±0.002	0.2	1.10± 0.003	0.273
TP	5179±93	1.8	2598±85	3.27

n=3

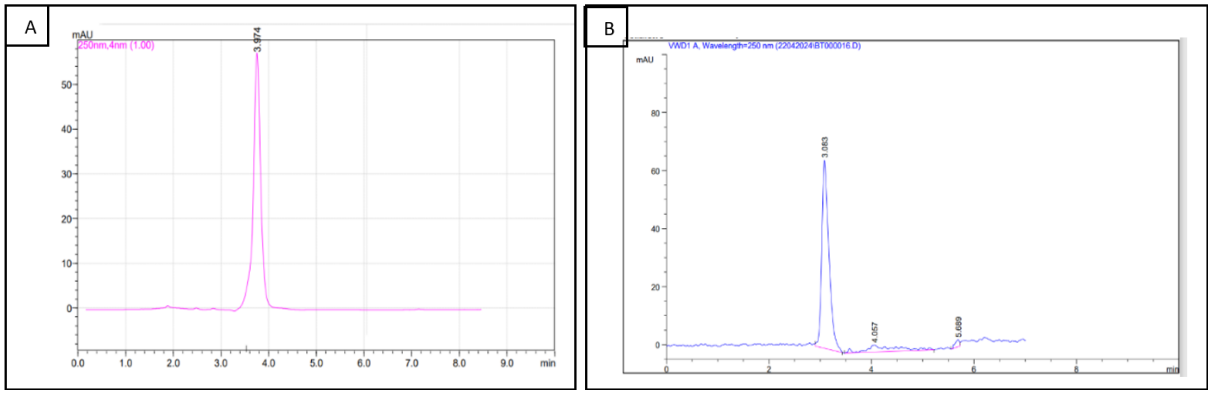

Fig. 5: HPLC chromatograms of (A) Bosutinib in mobile phase (B) BNCs Chromatographic conditions were injection volume 20 µl; flow rate 0.7 ml/min; temperature 25 °C; run time 10 min; detection wavelength 250 nm**

Table 8: Recovery study

Concentration (µg/ml)	Standard bosutinib	BNCs
	% Recovery	% Recovery
4.5	99.16±0.17	97.06±0.39
6	98.90±0.31	97.78±0.11
7.5	99.57±0.03	98.89±0.09

All the values are expressed as mean±SD, n=3

Table 9: Repeatability and intermediate precision

Concentration (µg/ml)	Inter Day				Intra Day			
	Standard bosutinib		BNCs		Standard bosutinib		BNCs	
	Mean±SD	%RSD	mean ±SD	%RSD	mean ±SD	%RSD	mean ±SD	%RSD
2	99.31±0.09	0.215	99.03±0.12	0.317	98.86±0.23	0.185	97.83±0.39	0.317
6	98.91±10	0.186	97.87±07	0.279	98.75±31	0.165	96.21±13	0.279
10	99.10±0.32	0.149	98.90±0.27	0.208	98.01±0.05	0.238	98.05±0.08	0.208

n=3

The standard deviation of the response and the slope obtained from linear regression of the calibration curve were used to calculate the LOD and LOQ within acceptable precision and accuracy. The minimum quantity of bosutinib and BNCs that can be found, or LOD and LOQ in Methanol: Ortho Phosphoric, Acid (pH 2.7) in a ratio of 80:20, For n = 3, the corresponding values were 38.95±0.99 ng/ml, 38.11 ±0.85 ng/ml, and91.19±0.35 ng/ml, 95.51±0.44 ng/ml [13, 19, 20].

CONCLUSION

In conclusion, this research developed and validated new analytical techniques for assessing and characterizing bosutinib and BNCs, Utilizing UV-visible spectroscopy for quantification, it was established that bosutinib dissolves well in methanol, allowing for robust calibration curves for both pure bosutinib and bosutinib-loaded nanocarriers. The methods demonstrated high precision and accuracy over a broad concentration range, with the detection limits confirming their sensitivity. Moreover, the development and validation of HPLC-based quantification methods for bosutinib, both in its pure form and when loaded into nanocarriers, were successful. These methods displayed excellent linearity, precision, and accuracy, meeting the rigorous criteria outlined in ICH Q2(R1) guidelines. The thorough validation of both UV-visible spectroscopy and HPLC methods highlights their appropriateness for routine analysis of bosutinib-loaded nanocarriers, thereby facilitating further research and development therapy.

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Nil

AUTHORS CONTRIBUTIONS

Experimentation and data analysis were done by Mr. Rishabh Agade, Manuscript drafting and experimentation work was carried out by Mr. Ujban Hussain, the concept for the work was proposed by Mr. Sagar Trivedi, this work was carried out under the supervision of Dr. Mrs. Veena Belgamwar.

CONFLICT OF INTERESTS

The authors declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships.

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