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Method Development and Validation of a UV Spectroscopic Approach for the Investigation of Ibrutinib in Bulk and Dosage Forms for Pharmaceuticals

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The aim of this research was to create a novel, quick, and precise UV-spectroscopic method for the quantification of Ibrutinib in both its pure form and in capsule dosage form. A straightforward ultraviolet spectrophotometric method was designed and validated based on several parameters, including linearity, precision, reproducibility, accuracy, robustness, ruggedness, as well as the LOD and LOQ. Methanol served as the diluent. The maximum absorbance was observed at 260 nm, and the linearity was discovered to be between 8.00-12.00 ìg/ml. The regression equation for Ibrutinib was y = 0.0533x + 0.0356, with a correlation coefficient (R^2) of 0.9998. The recovery percentage ranged between 98% and 102%. The RSD for both intraday and interday precision was under 2%. The LOD and LOQ were calculated to be 0.08 μg/mL and 0.24 μg/mL, respectively. The method validation was judged appropriate for regular quantitative analysis of Ibrutinib in both its pure and capsule dose forms in compliance with recommendations established by the International Conference on Harmonization (ICH).

Keywords: Ibrutinib, Method Development, Methanol, UV-Spectroscopy, Validation.

Ibrutinib, also referred to as PCI-32765, is a pioneering drug that irreversibly inhibits Bruton's Tyrosine Kinase (BTK), a key enzyme involved in Signaling by B-cell receptors. FDA approval allows adult patients with several hematological disorders—including Waldenstrom's macroglobulinemia (WM), marginal zone lymphoma (MZL), small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), and chronic graft-versus-host disease (cGVHD)—treatment with Ibrutinib.[2]

Ibrutinib received FDA approval in 2014 for chronic lymphocytic leukemia and in 2013 for mantle cell lymphoma. Additionally, it treats Waldenstrom macroglobulinemia, Chronic graft-versus-host disease, small lymphocytic lymphoma, marginal zone lymphoma, and chronic lymphocytic leukemia. By blocking the aberrant protein that gives cancer cells signals to proliferate, the medication slows the growth of cancer cells.[3-7] The chemical structure of Ibrutinib was displayed in Fig 1

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MATERIAL AND METHOD

Instrumentation

The absorbance of Ibrutinib solution was measured using Double beam UV- Visible spectrophotometer 550 Jasco. The instrument used in this study includes matched quartz cells integrated with Spectra Manager software for analysis. For measurement, an electronic balance was utilized. Additionally, pipettes and volumetric flasks made of borosilicate glass were employed in the experimental procedure.

Chemicals and reagents

Ibrutinib drug sample was purchased by V & S Laboratory. Nitib 140 mg capsule procured as Marketed formulation for estimation. All of the chemicals used were of analytical quality.

Drug stock solution preparation

Measured 10 milligrams of Ibrutinib and put it in a 20 ml volumetric flask. Methanol was added to the volume to achieve a 500-ppm concentration.

Preparation of working standard solution

Pipette out 0.4 mL of Ibrutinib stock solution and diluted up to volumetric flask of 20 mL with Methanol to achieved 10 PPM of Ibrutinib.

Preparation of calibration curve

Approximately 20.0 mg of Ibrutinib was measured and shifted into a 50 mL volumetric flask. Subsequently, 30 mL of methanol was incorporated, and the liquid was sonicated until Ibrutinib completely dissolved. After then, methanol was added to get the volume up to the 50 mL threshold.

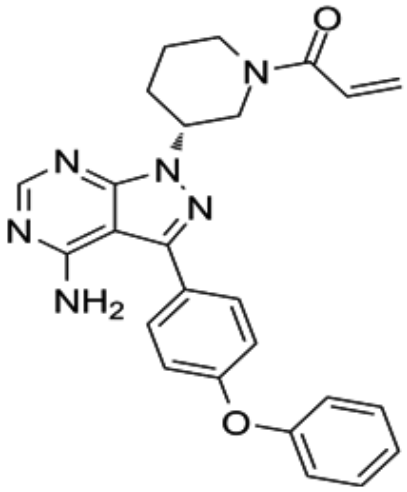

Fig. 1. Ibrutinib structure

0.5 ml of the standard stock solution was put into a 20 ml volumetric flask that had been pipetted out. After adding methanol to bring the volume up to the 20 mL level, the working concentration was 10 µg/mL. Five copies of standard medication solution were used to obtain data for the tests, and the results were documented appropriately.

Assay

Opened the 20 capsule shells and transfer their contents on butter paper and weigh it. Record the weight and calculated the average weight (235.3 mg) of filled contents of capsule.

A sample of powder material weighing 235.3 mg, corresponding to 100 mg of Ibrutinib, was moved to a 100-milliliter capacity flask that had been cleaned and dried. To this, following the addition of 70 mL of methanol, the solution underwent sonic for fifteen minutes while being shaken occasionally. Following sonication, the mixture was left to cool to ambient degree before Methanol was introduced to get the volume down to the 100 mL mark. The first 3-5 ml of the filtrate were discarded after the the mixture was strained using a Syringe filter, 0.45 µm. A concentration of 10 µg/mL of Ibrutinib was obtained by further diluting a 0.2 mL aliquot of the stock solution's filter with 20 mL of methanol. The resulting solution was analysed at 260 nm.

Method validation

The International Conference on Harmonization (ICH) criteria Q2 (R1) (ICH, 2005) were followed in the validation of the approach. [8] The parameters listed below were evaluated as part of the validation process:

Specificity

The ability to precisely measure the analyte while being surrounded by additional substances that might be there in the sample is called as specificity. The primary goal of assessing specificity is to ensure the "peak purity" of the target compound. Specificity was established by analysing a concentration of 10 µg/mL of the analyte repeatedly and quantifying the absorbance at a specific wavelength of 260 nm.

Linearity and Range

An analytical process's capacity to

produce outcomes from tests that are exactly proportionate to the analyte's concentration (amount) inside a specified range is understood as linearity. Linearity was evaluated by performing tests at 5 distinct levels of concentration, ranging from 80% to 120% of the test concentration.

Ibrutinib stock solution's linearity

10 mg of Ibrutinib was weighed and submerged within 20 mL of methanol. In order to achieve a 50 ppm concentration, this solution was then further diluted by moving 5 mL to a 50 mL volumetric flask and adding methanol until the mark was reached. The mean absorbance was computed after each concentration level was examined in triplicate. According to the results, a calibration curve was created by laying out the mean absorbance on the Y-axis versus the analyte concentration in µg/mL on the X-axis.

LOD and LOQ

Detection limit

The detection limit of a certain analytical procedure is the lowest concentration of analyte in a sample that can be recognized but isn't necessarily measured as an exact value.

Quantitation limit

The quantitation limit of a given analytical procedure is the lowest analyte concentration in a sample that can be quantitatively determined with suitable precision and accuracy.

According to the ICH Q2R1 guidelines, the Limits of Detection (LOD) and Limits of Quantification (LOQ) were determined using the calibration curve method. The following formulas were used to calculate the regression line's residual standard deviation (ó) and determine the LOD and LOQ:

LOD = (3.3 × ó) / S

LOQ = (10 × ó) / S

Where:

ó is the regression line's residual standard deviation

S is the regression line's slope

Accuracy (% Recovery)

The level of accord between the measured/ observed value and the acknowledged true or reference value is a measure of the analytical procedure's accuracy. Accuracy was assessed by preparing solutions at levels between 80% and 120% of the working concentration. Three replicas of each accuracy level were created to assess the procedure's reliability and precision across the specified concentration range.

Precision

Precision in Process of an analysis relates to the outcomes' consistency when multiple measurements are taken from identically uniform sample under the same conditions that are prescribed. Precision is categorized into two types: **Repeatability** and **Intermediate Precision**. In this study, precision was evaluated on the capsule test sample.

The precision of the method was assessed in terms of:

Repeatability: The consistency of results under the same conditions in a single run.

Intra-day precision: The disparity between measurements made on the same day

Inter-day precision: The variation in measurements taken on different days.

Repeatability

Sample solution preparation (6 samples prepared)

A sample of powder material weighing 235.3 mg (Average weight), equal to 100 milligrams of Ibrutinib, was moved to a Flask with a volume of 100 mL that had been cleaned and dried. To this, Following the addition of 70 mL of methanol, the liquid was vibrated (sonication) for 15 min. while being shaken occasionally. After sonication, after letting the solution settle to R.T., Methanol was introduced to get the volume down to the 100 mL mark. After that, after that, the mixture went through a 0.45 µm nylon filter, and the first three to five milliliters of filtrate were discarded. A 0.2 mL aliquot of the filtered stock solution was further diluted to 20 mL with methanol, resulting in a concentration of 10 µg/mL of Ibrutinib. The absorbance of the solution was recorded at 260 nm. Six samples were prepared and analysed.

Intermediate Precision (Ruggedness)

Inter-day precision is evaluated by conducting the analysis on a separate day from evaluate the outcomes' repeatability. The samples were prepared similarly to how they were for the test of repeatability, with six samples prepared on another day and by another analyst. This helps ensure the method's consistency and reliability across different conditions.

Description	Observation
Blank	No interference at Absorption maxima of Ibrutinib due to blank
Placebo	No interference at Absorption maxima of Ibrutinib due to blank placebo
Standard solution	Absorption maxima of Ibrutinib standard solution found at 260 nm
Test Solution	UV spectrum of Test Solution found concordant to that of standard solutionAbsorption maxima of Ibrutinib test solution found at 260 nm

Robustness

The robustness of an analytical procedure demonstrates its dependability under normal operating conditions and its capacity to tolerate small but deliberate changes in method parameters.

RESULTS

Analytical Method Validation Parameters

According to ICH criteria, the procedure was validated (Q2 (R1).

Specificity

Blank, placebo, Ibrutinib standard and capsule test sample solutions prepared and analysed

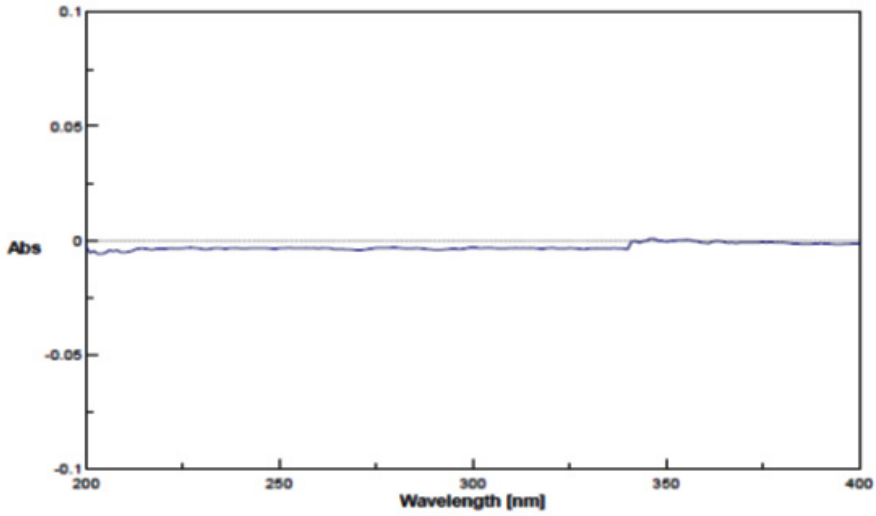

Fig. 2. Typical UV-spectrum of Blank solution

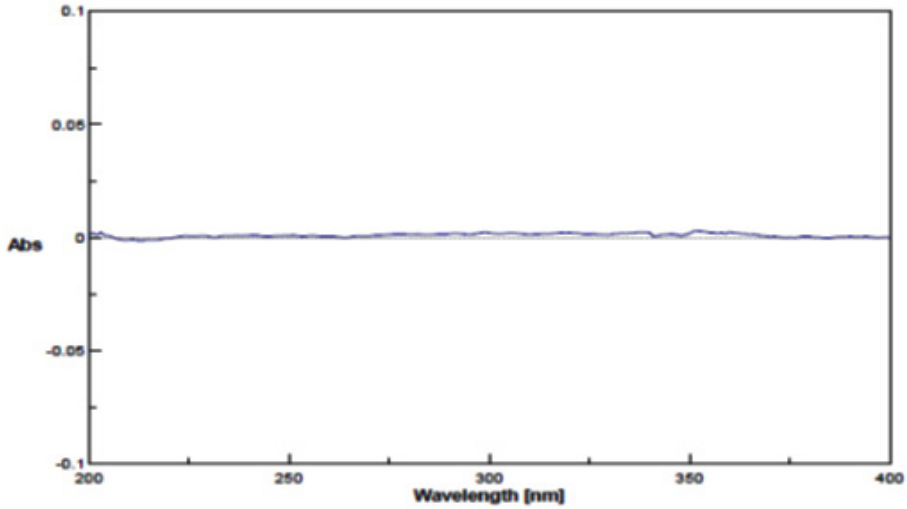

Fig. 3. Typical UV-spectrum of Placebo solution

to prove the specificity nature of the method. (Scanned each solution from 400 nm to 200 nm)

Total 5 gm of placebo prepared. Weighed 135.3 mg of placebo material (Which is equivalent to 100 mg of Ibrutinib) and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 15 minutes with intermittent shaking. After 15 minutes allowed the solution to cool at room temperature and made volume up to the mark with methanol. Filtered the solution through 0.45 µ Nylon filter discarding 3-5 mL of initial filtrate. Further dilute 0.2 ml of filtered

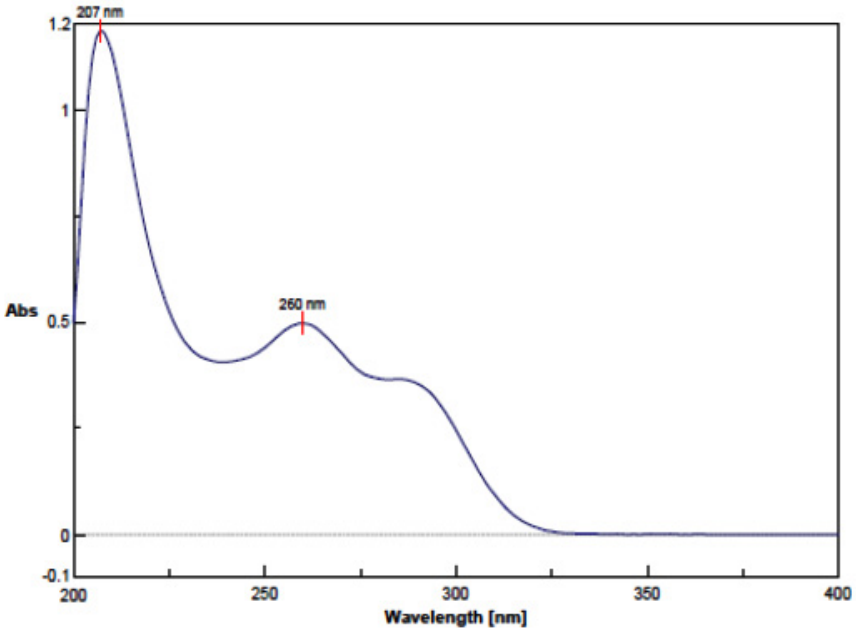

Fig. 4. Typical UV-spectrum of Standard solution

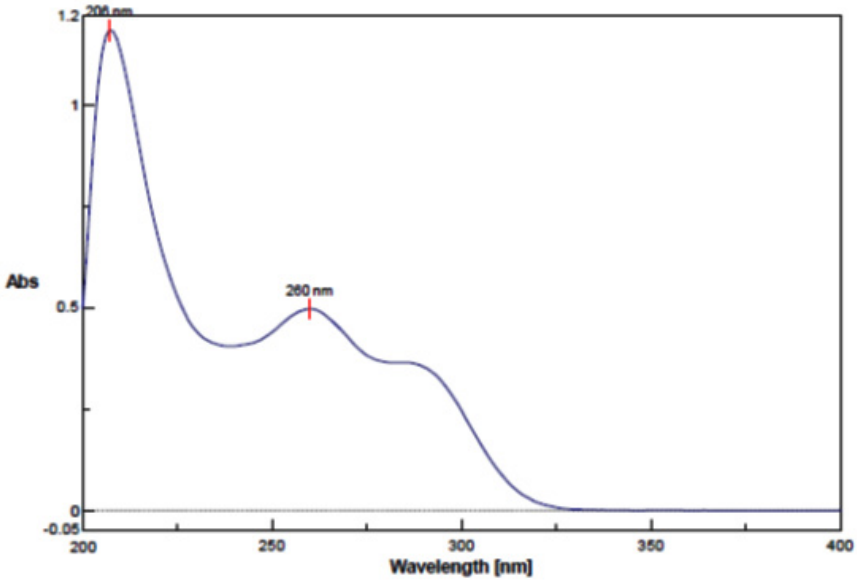

Fig. 5. Typical UV-spectrum of Test sample solution

stock solution to 20 ml with methanol, scanned from 400 nm to 200 nm.

Sample preparation of Marketed test sample

Weighed the powder material (235.3 mg) equivalent to 100 mg of Ibrutinib and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 15 minutes with intermittent shaking. After 15 minutes allowed the solution to cool at room temperature and made volume up to the mark with methanol. Filtered the solution through 0.45 µ Nylon filter discarding 3-5 mL of initial filtrate. Further diluted 0.2 ml of filtered stock solution to 20 ml with methanol. (10 mcg of Ibrutinib), scanned from 400 nm to 200 nm.

Blank and placebo were not having interference at absorption maxima of Ibrutinib. UV spectrum of Test Solution found concordant to that of standard solution. Absorption maxima of Test sample found within ± 2 NM to that of Absorption maxima of Standard solution. Hence developed UV method passed the criteria for specificity.

Linearity and Range

The spectra of Ibrutinib are as shown in Figure 2. The linearity equation for Ibrutinib was

Table 1. Linearity data of ibrutinib

Level	Conc (µg/mL)	Absorbance	Mean	% RSD
80%	8.00	0.3927	0.3925	0.170
		0.3931		
		0.3918		
90%	9.00	0.4416	0.4425	0.185
		0.4432		
		0.4427		
100%	10.00	0.4962	0.4973	0.192
		0.4978		
		0.4979		
110%	11.00	0.5511	0.5518	0.118
		0.5524		
		0.5518		
120%	12.00	0.6045	0.6045	0.108
		0.6052		
		0.6039		

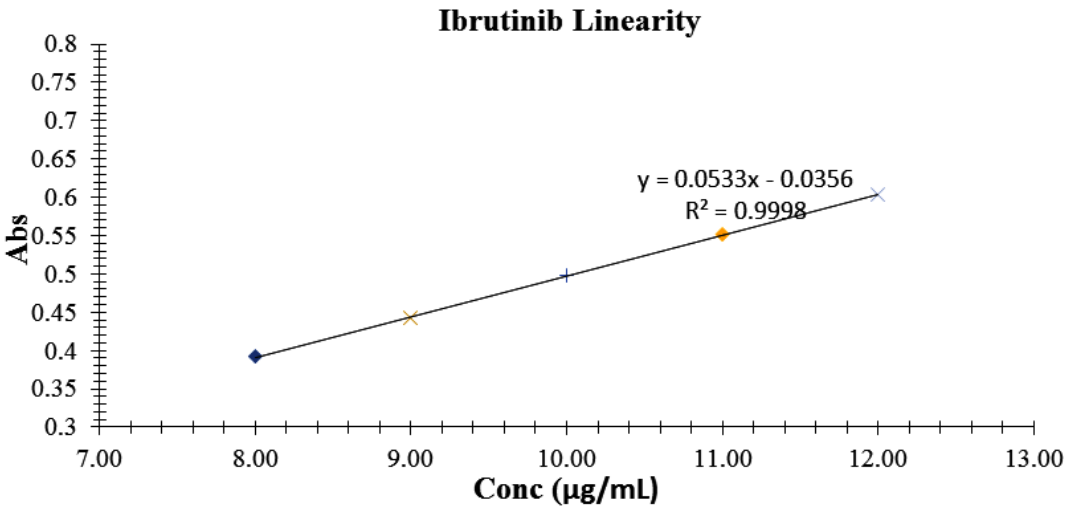

Fig. 2. Curve of Ibrutinib calibration

discovered to be Y = 0.05333x - 0.03558, with a correlation coefficient of 0.99989. Based on the linearity data, the coefficient of correlation (R^2) was found to be less than 2, indicating that the results fall within the acceptable limits.

The linearity outcomes are provided in Table 1, and In Figure 2, the comparable graph is displayed.

Accuracy

Each sample's mean percentage recovery should be between 98 and 102%. The RSD should not exceed 2.0%, indicating that the results are within acceptable limits. Every one of the three levels, the analytical procedure's recovery was determined to fall comfortably within the permissible range. The percentage recovery was not

Table 2. Accuracy Results of Ibrutinib

Level (%)	Absorbance	Ibrutinib Added conc. (µg/mL)	Recovered conc. (µg/mL)	% Recovery	Mean Recovery	% RSD
80	0.3951	8.02	7.95	99.13	100.33	1.055
	0.4035	8.03	8.12	101.12		
	0.4027	8.04	8.10	100.75		
100	0.4982	10.02	10.02	100.00	100.07	1.300
	0.5034	9.98	10.12	101.40		
	0.4915	10.01	9.89	98.80		
120	0.6045	12.05	12.16	100.91	99.91	1.091
	0.5976	12.01	12.02	100.08		
	0.5908	12.03	11.88	98.75		

Table 3. Intraday precision Results

Repeatability	Sample	Test Sample (mg)	Absorbance	% Assay
	Sample 1	235.2	0.485	97.58
	Sample 2	235.8	0.4892	98.18
	Sample 3	234.8	0.4791	96.56
	Sample 4	235.2	0.4805	96.68
	Sample 5	235.8	0.4772	95.77
	Sample 6	234.7	0.4872	98.23
	Mean			97.17
	STD DEV			0.9883
	% RSD			1.017
Intermediate precision (Inter-Day)	Sample 1	235.8	0.4851	97.35
	Sample 2	235.1	0.4892	98.47
	Sample 3	235.5	0.4903	98.52
	Sample 4	234.9	0.4788	96.46
	Sample 5	236.5	0.4868	97.41
	Sample 6	235.7	0.4908	98.54
	Mean			97.79
	STD DEV			0.8561
	% RSD			0.875
Repeatability Plus Inter-day	Mean			97.479
	STD DEV			0.9400
	% RSD			0.964

affected by changes in the analyte concentration. Table 2 displays the accuracy outcomes.

Precision

Precision is categorized into two types: Repeatability and Intermediate Precision. These tests were performed on the capsule test sample to assess the consistency and dependability of the analytical technique under both the same conditions (repeatability) and across different conditions (intermediate precision).

Repeatability

A powder material weighing Average weight 235.3 mg, equal to 100 mg of Ibrutinib, was poured into a volumetric flask that holds 100

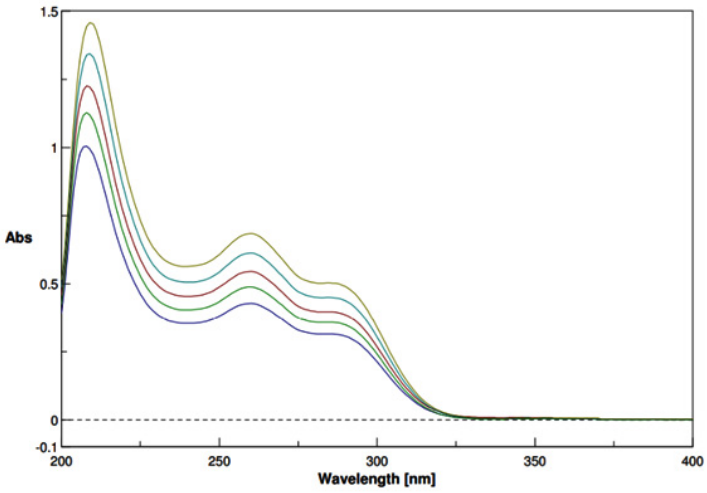

Fig. 3. UV Spectra of linearity

Table 4. Results of Change in sonication time by ± 5 minutes (10 & 20 minutes)

	-5 min (10 min.)				+5 min (20 min.)		
Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value	Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value
Sample 1	0.4837	97.07	0.19	Sample 1	0.4821	97.04	0.46
Sample 2	0.4853	97.64		Sample 2	0.4782	96.38	
Mean	97.36			Mean		96.71	
STD DEV	0.4031			STD DEV		0.4667	
% RSD	0.414			% RSD		0.483	

Table 5. Results of Test samples by change in + 2 nm wavelength

	-2 nm				+2 nm		
Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value	Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value
Sample 1	0.4765	97.07	0.25	Sample 1	0.4768	96.97	0.16
Sample 2	0.4812	97.64		Sample 2	0.4816	97.69	
Mean	97.33			Mean	97.33		
STD DEV	0.5140			STD DEV	0.5140		
% RSD	0.528			% RSD	0.528		

Table 6. Assay of Nitib 140 mg capsules

Sample	Absorbance	% Assay	Mean Assay
Sample 1	0.4838	97.09	97.39
Sample 2	0.4859	97.68	

Table 7. Review of validation parameter

Parameters	Obtained values
Maximum absorbance (λ max)	260 nm
Linearity (υg/ml)	8-12 ìg/ml
Intercept (c)	-0.03558
Slop (m)	0.05333
Intra-Day Precision (% RSD)	1.017
Inter-Day Precision (% RSD)	0.875
Recovery (%)	100.10 %
LOD	0.08 µg/mL
LOQ	0.24 µg/mL

mL that had been cleaned and dried. Following the addition of 70 mL of methanol, the liquid spent 15 minutes being sonicated while being shaken occasionally. After sonication, the solution was After allowing it to cool to ambient temperature, methanol was added to get the volume up to 100 mL. After that, the solution was passed via a nylon filter with a 0.45 µm opening, and the first three to five milliliters of filtrate were discarded. A 0.2 mL aliquot of the filtered the initial mixture was further diluted to 20 mL with methanol, resulting in a 10 µg/mL concentration of Ibrutinib. The absorbance of the solution was recorded at 260 nm. Six samples were prepared for analysis.

Intermediate Precision (Ruggedness)

Inter-day precision is assessed by performing the analysis Some other day to evaluate the results' reproducibility. The samples were prepared similarly to how they were for the test of repeatability, with six samples prepared on another day and by another analyst. This strategy guarantees that the process yields dependable and consistent outcomes under various personal and circumstance scenarios.

Intraday precision

The percentage RSD of the precision studies was discovered to be below 2%, indicating that the results are within the acceptable range and demonstrating good precision concerning the analytical approach. The intraday precision the findings were displayed in Table No. 3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

ó = 0.00126 (Residual standard deviation of a regression line)

s = 0.05333 (Slope)

Detection limit (LOD):

LOD = 3.3 ó / S

LOD = 3.3 x 0.00126 / 0.05333

LOD = 0.08 µg/mL

Quantitation limit (LOQ):

LOQ = 10 ó / S

LOQ = 10 x 0.00126 / 0.05333

LOQ = 0.24 µg/mL

Robustness

Test solution and Standard solution were analysed under various circumstances, as illustrated below.

a) Changes in Sonication time for test sample preparation by ±5 min

b) Change in wavelength (± 2 nm)

Changes in Sonication time for test sample preparation by ± 5 min

Two samples prepared by change in this parameter.

Changes in wavelength by ± 2 nm

First two samples of Precision study analyzed at this wavelength and calculated its assay value. Abs difference calculated for assay value w.r.t. Precision assay value (Mean value).

Results of change in wavelength by ± 2 nm

Difference between precision assay value and robustness assay value is not more than 2.0. it was concluded that the difference between precision assay value and robustness assay value was discovered to be substantially within the bounds, and the analytical technique proved reliable.

Assay

Marketed test sample (Nitib 140 mg capsule) was prepared twice (Average weight 235.3 mg) equivalent to 100 mg of Ibrutinib, then the purity percentage was found to be 97.39%. The amount of drug involved extracted was calculated from the sample solutions to be within the allowable 90–110% range, as stated on the label. Table 6 displays the assay data. ICH recommendations were adhered to when validating the devised approach, and an overview of the results is provided in Table 7.

DISCUSSION

An ultraviolet spectrophotometric method was designed and validated based on several parameters, including linearity, precision, reproducibility, accuracy, robustness, ruggedness, as well as the LOD and LOQ. The method validation was judged appropriate for regular quantitative analysis of Ibrutinib in both its pure and capsule dose forms in compliance with recommendations established by the International Conference on Harmonization (ICH). The absorbance of Ibrutinib solution was measured using Double beam UV- Visible spectrophotometer 550 Jasco. The instrument used in this study includes matched quartz cells integrated with Spectra Manager software for analysis.

The developed method is specific as no interference from excipients was observed. The ëmax of the drug remained unchanged under different conditions, confirming its selectivity. Each concentration level was examined in triplicate for linearity, and the mean absorbance was determined. The analyte concentration in µg/mL was displayed on the X-axis of the calibration curve, while the mean absorbance was displayed on the Y-axis. With a correlation value of 0.99989 less than 2, a significant linear relationship between absorbance and concentration was established in the selected range of 8–12 µg/mL, suggesting that the results are within acceptable bounds.

The average recovery percentage for each sample should be between 98 and 102%. The results should fall within acceptable bounds if the RSD is less than 2.0%. Variations in the analyte concentration had no effect on the recovery %.

The % RSD for repeatability, intraday, and interday precision was less than 2%, proving the method's exceptional precision and showing that the results are within an acceptable range with respect to the analytical technique. According to calculations, the LOD and LOQ were 0.08 µg/mL and 0.24 µg/mL, respectively. The analytical method was robust, and the difference between the precision and robustness assay values was found to be well within the bounds.

The method's development and validation in compliance with ICH requirements ensured its accuracy, precision, and robustness for Ibrutinib analysis.

CONCLUSION

Statistical analysis revealed that the procedure was accurate and exact, proving its applicability and dependability for regular Ibrutinib analysis.

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Conflict of interest

The authors declare that there is no conflict of competing financial interests.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Kishori Laxman. Deore: Conceptualization, Methodology, Writing – Original Draft, Data Collection, Analysis, Writing – Review & Editing; Gokul Shravan Talele: Visualization, Supervision.

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