Analytical method development and validation for the quantitative analysis of Ibrutinib in API form and marketed pharmaceutical dosage form by using RP-HPLC

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Abstract

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Ibrutinib in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (35:65) v/v as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The runtime under these chromatographic conditions was less than 8 min. The retention time of Ibrutinib was found to be 2.276min. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Ibrutinib in bulk and marketed pharmaceutical dosage form dosage form.

Keywords: Ibrutinib, RP-HPLC, Validation, Accuracy, Precision, ICH Guidelines

Introduction

Ibrutinib is a member of the class of acrylamides that is (3R)-3-[4-amino-3-(4-phenoxyphenyl) pyrazolo [3, 4-d] pyrimidin-1-yl]piperidine in which the piperidine nitrogen is replaced by an acryloyl group. A selective and covalent inhibitor of the enzyme Bruton's tyrosine kinase, it is used for treatment of B-cell malignancies. It has a role as an EC 2.7.10.2 (non-specific protein-tyrosine kinase) inhibitor and an antineoplastic agent. It is a pyrazolopyrimidine, an aromatic amine, aromatic ether, a member of acrylamides, an N-acylpiperidine and a tertiary carboxamide.Ibrutinib[1] is a small molecule that acts as an irreversible potent inhibitor of Burton's tyrosine kinase. It is designated as a targeted covalent drug and it presents a very promising activity in B cell malignancies. Ibrutinib was developed by Pharmacyclics Inc and in November 2013 was FDA-approved for the treatment of mantle cell lymphoma. Later, in February 2014, Ibrutinib was approved for the treatment of chronic lymphocytic leukemia and it is also indicated for the treatment of patients with Waldenström's Macroglobulinemia. Ibrutinib[2] has also been approved by the EMA for the treatment of chronic lymphocytic leukemia and mantle cell lymphoma. Ibrutinib was approved for use in chronic graft versus host disease (cGVHD) in August 2017 which was later approved to be used in children, making it the first FDA-approved cGVHD treatment for kids 1 year and older after failure of one or more lines of systemic therapy. Ibrutinib [3] is a Kinase Inhibitor. The mechanism of action of Ibrutinib is as a Protein Kinase Inhibitor. The IUPAC Name of Ibrutinib is 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl) pyrazolo [3, 4-d] pyrimidin-1-yl] piperidin-1-yl] prop-2-en-1-one. The Chemical Structure of Ibrutinib is as follows

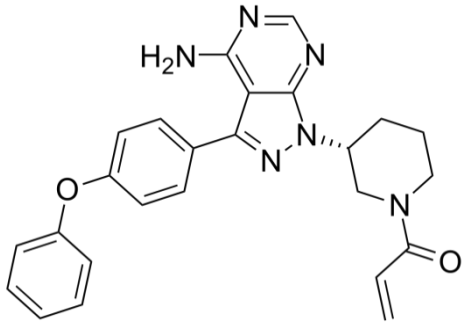

Fig 1: Chemical Structure of Ibrutinib

Materials and Methods

Instruments Used

Table 1: Instruments used

S.No	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals Used:

Table 2: Chemicals used

S.No.	Chemical	Brand Names
1	Ibrutinib	Synpharma Lab, Dilsuknagar
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Method Development

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate[4] to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Ibrutinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[27].

Mobile Phase Optimization

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase[5]was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

Optimization of Column

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution [6] at 1ml/min flow.

Preparation of Buffer and Mobile Phase

Preparation of Potassium Dihydrogen Phosphate (KH2PO4) Buffer (pH-3.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration [7] and ultra-sonication.

Preparation of Mobile Phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent [8].

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ibrutinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the aboveIbrutinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Weight 10 mg equivalent weight ofIbrutinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Ibrutinib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay [9, 10] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (6ppm of Ibrutinib)

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (8ppm of Ibrutinib)

Take 0.8ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (10ppm of Ibrutinib)

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator [11].

Preparation of Level – IV (12ppm of Ibrutinib)

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (14ppm of Ibrutinib)

Take 0.14ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure

Inject each level into the chromatographic system[12] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Ibrutinib Product Solution for Precision

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ibrutinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Analyst 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For Preparation of 50% Standard Stock Solution

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Ibrutinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ibrutinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 150% Standard Stock Solution

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Ibrutinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Ibrutinib and calculate the individual recovery and mean recovery values.

Limit of Detection and Limit of Quantification (LOD & LOQ)

Preparation of 0.95µg/ml Solution (For LOD)

Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock

solution)
Further pipette 0.0095ml of the above Ibrutinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 2.9µg/ml Solution (For LOQ)
Accurately weigh and transfer 10 mg of Ibrutinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.029ml of the above Ibrutinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Ibrutinibworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Ibrutinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition
The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead (35:65), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Results and Discussion

Method Development

Optimized Chromatographic Conditions
Mobile phase ratio: Methanol: Phosphate Buffer (35:65) V/V
Column: Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature: Ambient
Wavelength: 235nm
Flow rate: 1ml/min
Injection volume: 10µl
Run time: 8min

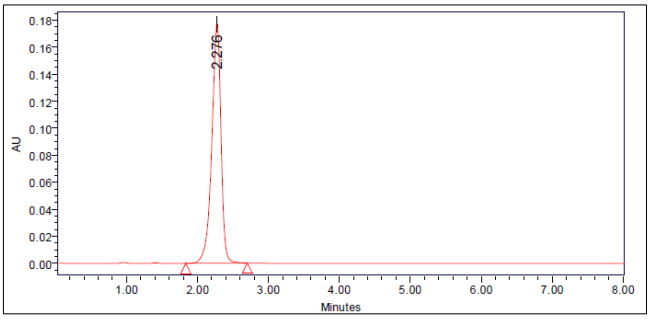

Fig 2: Optimized Chromatographic Condition

Method Validation
The method[13] was validated by following the ICH and USP guidelines for system suitability, linearity, accuracy, precision, specificity and limit of detection (LOD).

System Suitability
The system suitability [14] was checked by analyzing the repeatability of retention time, tailing factor and theoretical plates of the column.

Table 3: Results of System Suitability for Ibrutinib

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ibrutinib	2.277	1652847	185647	6589	1.24
2	Ibrutinib	2.277	1653658	186254	6587	1.26
3	Ibrutinib	2.267	1654521	185475	6584	1.28
4	Ibrutinib	2.265	1653564	186594	6582	1.29
5	Ibrutinib	2.277	1658745	185684	6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
%RSD			0.142371			

Specificity
The ICH documents define specificity [15] as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.
Analytical method was tested for specificity to measure accurately quantitates Ibrutinib in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

$$= 99.40\%$$

The % purity [16] of Ibrutinib pharmaceutical dosage form was found to be 99.40%.

Linearity
To evaluate the linearity [17] of this method, five standard solutions covering the range of 6 to 14µg/ml were analyzed using the method. The plot of peak area ratio of drug and against respective concentration of the drug was found to be linear in the range as shown in figure -3. The regression equation [18] was found to be Y= 185008X-16179 and the coefficient of determination R^2 of the standard curve was found to be 0.9996.

Chromatographic Data for Linearity Study:

Table 4: Data for Linearity of Ibrutinib

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

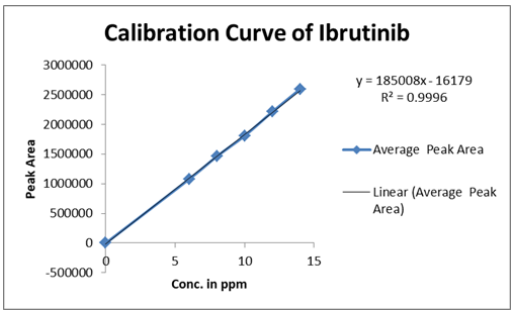

Fig 3: Linearity Curve of Ibrutinib

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Ibrutinib is a straight line.

Y = mx + c

Slope (m) = 18500

Intercept (c) = 16179

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient [19] is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 0.16179. These values meet the validation criteria.

Precision

The precision [20] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [21].

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD [22].

Table 5: Results of Repeatability for Ibrutinib

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ibrutinib	2.293	1658954	186958	1.26	6785
2	Ibrutinib	2.276	1658745	187548	1.27	6854
3	Ibrutinib	2.286	1659865	189854	1.26	6852
4	Ibrutinib	2.277	1653254	186985	1.25	6784
5	Ibrutinib	2.280	1654781	189542	1.24	6895
6	Ibrutinib	2.293	1661324	187586	1.28	6965
Mean			1657821			
Std. Dev			3120.433			
%RSD			0.188225			

Intermediate Precision

Analyst-1:

Table 6: Results of Intermediate Precision for Ibrutinib

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ibrutinib	2.274	1678541	186589	6587	1.26
2	Ibrutinib	2.258	1685985	186598	6321	1.26
3	Ibrutinib	2.267	1685745	186985	6385	1.25
4	Ibrutinib	2.270	1685987	187854	6580	1.26
5	Ibrutinib	2.264	1698526	187549	6721	1.27
6	Ibrutinib	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std. Dev.			6463.466			
%RSD			0.383182			

Analyst2:

Table 7: Results of Intermediate precision Analyst 2 for Ibrutinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USPP late count	USP Tailing
1	Ibrutinib	2.277	1665847	167481	6854	1.25
2	Ibrutinib	2.255	1658989	167854	6785	1.26
3	Ibrutinib	2.265	1659845	167895	6854	1.24
4	Ibrutinib	2.255	1665964	167854	6895	1.26
5	Ibrutinib	2.253	1659863	168585	6459	1.25
6	Ibrutinib	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std.Dev.			3501.766			
%RSD			0.210601			

Accuracy: As per the ICH guideline, accuracy is inferred once linearity, specificity and precision of the method are established. This approach was followed to establish the accuracy [23] of the method and the method was found to be

accurate.

Table 8: The Accuracy Results for Ibrutinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

Limit of Detection

The detection limit [24] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

= 0.95µg/ml

Quantitation Limit

The quantitation limit [25] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

= 2.9µg/ml

LOD	0.95925344
LOQ	2.906828605

SE of Intercept = Excel Function (Data Analysis → Regression)

SD of Intercept = SE of Intercept * √N

LOD = 3.3 * (SD of Intercept/Slope)

LOQ = 10 * (SD of Intercept/Slope)

Robustness

The robustness [26] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ibrutinib. The method is robust only in less flow condition. The standard of Ibrutinibwas injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 9: Results for Robustness

Parameter Used For Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

Summary and Conclusion

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 235nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry ODS C_{18} (4.6×250mm, 5µm) because it was giving good peak.

Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: Phosphate Buffer pH-3.6 in the ratio of 35:65 v/v was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery.

Run time was selected to be 8min because analyze gave peak around 2.276 and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range of 6-14ppm of the Ibrutinib target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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