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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE DETERMINATION OF IBRUTINIB IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A novel, simple, accurate and precise Zero order spectroscopic method was developed and validated for the determination of Ibrutinib in bulk and tablet dosage forms and has an absorption maximum at 258 nm in Acetonitrile. The Linearity was found to be in the concentration range of 2-12 µg/ml and the correlation coefficient was found to be 0.9999 and it has showed good linearity, reproducibility, precision in this concentration range. The regression equation was found to be Y = 0.0548X+ 0.0007. The % recovery values were found to be within 100.02 -100.43 % showed that the method was accurate. The LOD and LOQ were found to be 0.0957 and 0.29 µg/ml, respectively. The % RSD values were less than 2%. The method has been validated according to ICH guidelines for linearity, accuracy, precision, robustness, ruggedness. Limit of detection and limit of quantitation. Proposed method was successfully applied for the quantitative determination of Ibrutinib in bulk and tablet dosage form.

KEYWORDS: Ibrutinib, UV- Spectroscopy, Acetonitrile, Accuracy.

INTRODUCTION

Ibrutinib belongs to a family of drugs known as kinase inhibitors.[1] Ibrutinib, a United States Food and Drug Administration approved drug, chemically known as 1-[(3R)-3 -[4- Amino-3-(4-phenoxyphenyl)-1H-pyrazolo [3, 4-d] pyrimidin-1-yl] piperidin-1-yl] prop- 2-en-1-one is a white to off-white solid powder soluble in polar solvents like acetonitrile, methanol and water. It is irreversible and elective small molecule which is used in the management of patients with chronic lymphocytic leukemia by binding perpetually to a protein, Bruton's tyrosine kinase (BTK), that inhibits B cell antigen receptor (BCR) signaling in human B cells via specific active-site occupancy2 It works by stopping the function of the abnormal protein that sends a proliferation signal to cancer cells.[1] It is useful in the treatment of Waldenstrom's macro globulinemia, lymphocytic leukemia and second-line treatment for marginal zone lymphoma, chronic graft versus host disease and mantle cell lymphoma.[3]

Fig.1: Chemical structure of Ibrutinib.

It has a molecular formula of $C_{25}H_{24}N_6O_2$ and molecular weight of 440.5g/mol. It has the structural formula (Fig.1).[8]

The literature survey reveals that various analytical methods have been developed such as Stability indicating HPLC[13], UV spectroscopy methods[9-11] for the determination of ibrutinib present in combinational pharmaceutical formulations and Comparative purity study of UV& FTIR Techniques[12] for the determination of Ibrutinib tablets. Most of the reported methods are using several solvents, expensive reagents and often time-consuming. Because of simplicity of UV spectrophotometry and also precise, reliable, minimum solvent usage and requires less analysis time, it is widely used for the determination of drug content in bulk and tablet products.

A detailed review of the literature regarding the existing methods revealed that there is a need for the development of the spectrophotometric method, which is simple for the determination of ibrutinib present in Bulk and tablet dosage forms. An effort was made in the present method to develop a novel, simple, sensitive, accurate, reliable and reproducible with minimum Relative Standard Deviation (RSD) values for the determination of ibrutinib in Bulk and tablet dosage forms.

MATERIALS AND METHODS

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Ibrutinib was given as a gift sample by Pharma industry Bangalore. Tablets of Ibrutinib were procured from local market.

Solvent: Acetonitrile is used as a solvent.

Selection of analytical wavelength: Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra obtained was showing the absorption maxima at 258 nm, as the wavelength for detection.

Preparation of standard stock solution: 100mg of Ibrutinib was weighed accurately transferred into 100 ml of volumetric flask and diluted in Acetonitrile up to the mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml into 10 ml individual volumetric flask and diluted in Acetonitrile up to the mark, this gives 2, 4, 6, 8, 10 and 12µg/ml concentration.

Preparation of sample solution: 20 tablets of Ibrutinib marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Ibrutinib was transferred into 100ml volumetric flask then it was diluted with Acetonitrile and make up to the mark.

METHOD AND VALIDATION

The method was validated according to the ICH guidelines.[15-17]

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity

Linearity shows how well the response of the method changes in proportion to the concentration of the drug within a given range. In other words, when the concentration increases, the absorbance should also increase in a consistent and predictable manner. The linearity was established in the range of 2-12µg/ml was measured at 306nm and absorbance values are shown in table 1. The calibration curve was prepared by plotting graph against the concentration vs absorbance and therefore the graph shown in Fig-3 statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in table-2.

Precision

Precision indicates how close the results are when the same sample is tested multiple times under similar conditions. If the variation between repeated measurements is very small, the method is considered precise. Precision was established by intra-day and inter-day was determined by analysing the same concentration for six times in a same day. Inter-day precision was analysing the same concentration daily for six days shown in table-3.

Accuracy

The accuracy of an analytical method says that closeness of test results obtained by that method of the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels at 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. Shown in table -4.

Ruggedness

Ruggedness evaluates whether the method gives consistent results when small changes are made, such as using different analysts or performing the test on different days. A rugged method produces similar results despite these minor variations. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (Table-5)

LOD and LOQ

LOD is the smallest amount of drug that the method can detect, even if it cannot measure it accurately. LOQ is the lowest amount of drug that can be measured accurately and precisely using the developed method.

LOD and LOQ were calculated by using following formula.
LOD = 3.3(σ/S) and LOQ = 10 (σ / S)

TABLES

Table 1: Results of calibration curve at 258nm by zero order derivative spectroscopy.

SL NO	Concentration in µg/ml	Absorbance ± Standard deviation*
1	2	0.111±0.0070
2	4	0.217±0.0141
3	6	0.333±0.0226
4	8	0.441±0.0291
5	10	0.549±0.0340
6	12	0.657±0.0404

**Average of six determinations.*

Table 2: Regression parameter for Ibrutinib by zero order derivative spectroscopy.

Regression parameter	Results
Range(µg/ml)	2-12
λ max(nm)	258
Regression Equation	Y= 0.0548x+0.0007
Slope(b)	0.0548
Intercept(a)	0.0007
Correlation coefficient(r^2)	0.9999
Sandell's equation	0.018
Limit of detection(µg/ml)	0.0957
Limit of quantitation(µg/ml)	0.29

Table 3: Determination of precision results for Ibrutinib at 258nm by zero order derivative spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
2	0.131±0.0013	0.99	0.126±0.00081	0.63
4	0.223±0.0022	0.98	0.222±0.0005	0.22
6	0.323±0.0031	0.95	0.324±0.0004	0.12
8	0.454±0.0040	0.88	0.455±0.0006	0.13
10	0.537±0.0042	0.78	0.537±0.0009	0.16
12	0.647±0.0045	0.69	0.648±0.0006	0.09

***Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Accuracy results for Ibrutinib at 258nm by Zero order spectroscopy.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	6	3	8.99	99.93±0.842	0.84
100	6	6	11.87	98.99±0.311	0.31
150	6	9	14.99	99.95±0.518	0.51

***Average of six determinations, **percentage relative standard deviation.*

Table 5: Determination of Ruggedness results for Ibrutinib at 258nm by Zero order spectroscopy.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.323	0.325
±Standard deviation*	0.0031	0.0032
%RSD	0.95	0.98

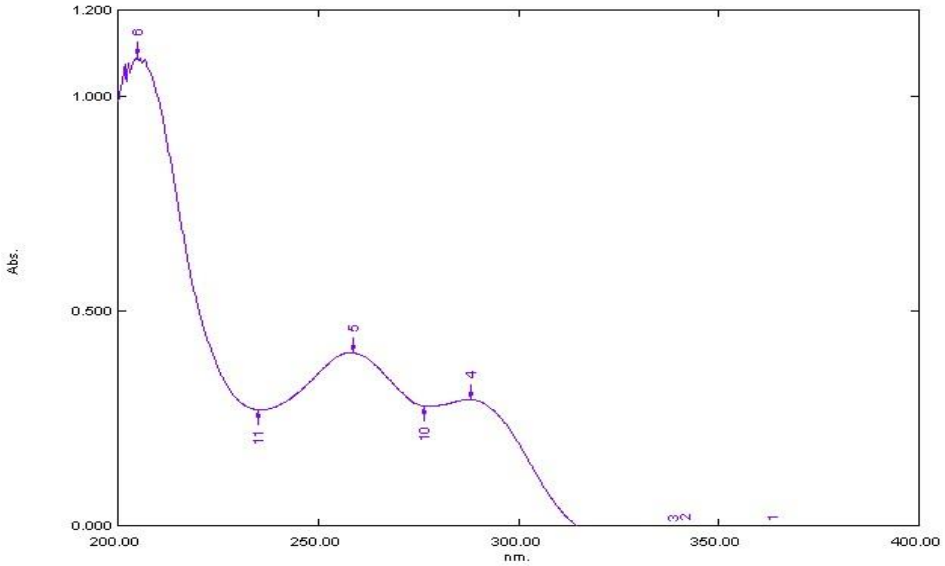
***Average of six determinations, **percentage relative standard deviation.*

Table 6: Determination of LOD and LOQ results for Ibrutinib at 258nm by Zero order derivative spectroscopy.

Sl. no	Parameters	Values
1	SD of Intercepts**	0.0016
2	Average of Slopes**	0.0548
3	LOD(3.3×SD of Intercepts/average of slopes)	0.0957
4	LOQ(10×SD of Intercepts/ average of slopes)	0.29

**Mean value obtained from six calibration curves.

FIGURES



Figures: Fig. 2: Zero order spectrum of Ibrutinib at 258nm.

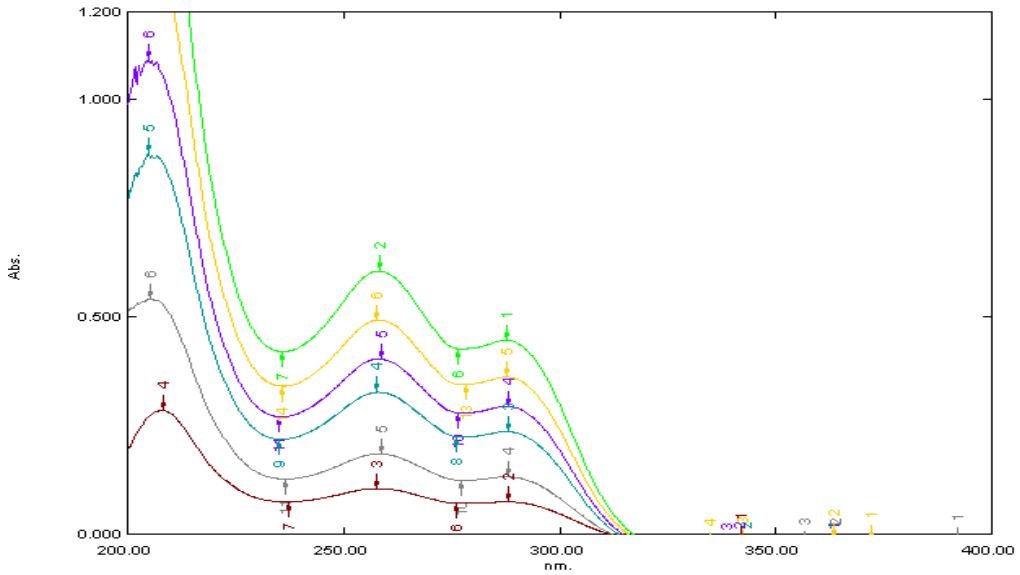


Fig. 3: Zero order overlay spectra of Ibrutinib showing absorbance at 258nm.

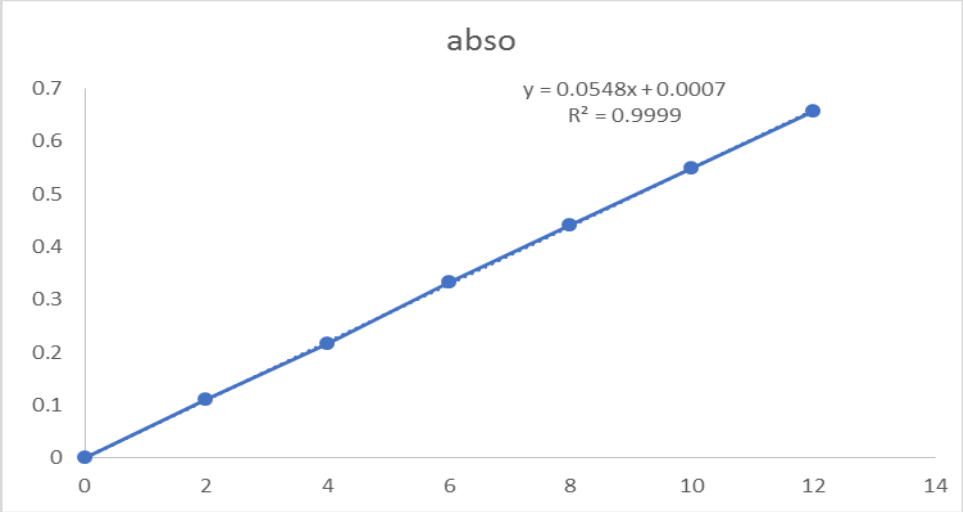


Fig.4: Calibration curve of Ibrutinib by zero order derivative spectroscopy.

CONCLUSION

As per ICH guidelines, the developed analytical method meets the acceptance criteria. It was concluded that this method is simple, specific, accurate, economical and sensitive and can be used for routine analysis of Ibrutinib in bulk drug and in tablet dosage forms.

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