UV-Spectrophotometric Method Development and Validation of Assay of Ceritinib Tablet Formulation

11.(B).1 Introduction

Ceritinib, 5-chloro-N4-[2-[(1-methylethyl)sulfonyl]phenyl]-N2-[5-methyl-2-(1-methyl ethoxy)-4-(4-piperidinyl)phenyl]2,4-pyrimidine diamine, is an anaplastic lymphoma kinase (ALK) inhibitor which induces complete tumour regression in a xenograft model of EML4-ALK-positive lung cancer. The alternative names of ceritinib are LDK 378, NVP-LDK 378, zykadia^{TM}. The IUPAC name of ceritinib (CRB) is 5-chloro-N^2-[2-isopropoxy-5-methyl-4-(4-piperidinyl)phenyl]-N^4-[2-(isopropylsulfonyl) phenyl]-2,4-pyrimidine diamine. The molecular formula of ceritinib is $C_{28}H_{36}ClN_5O_3S$ and its molecular mass is 558.14 g.mol^{-1}. The chemical structure of ceritinib was shown in **Fig 11.B.1.**

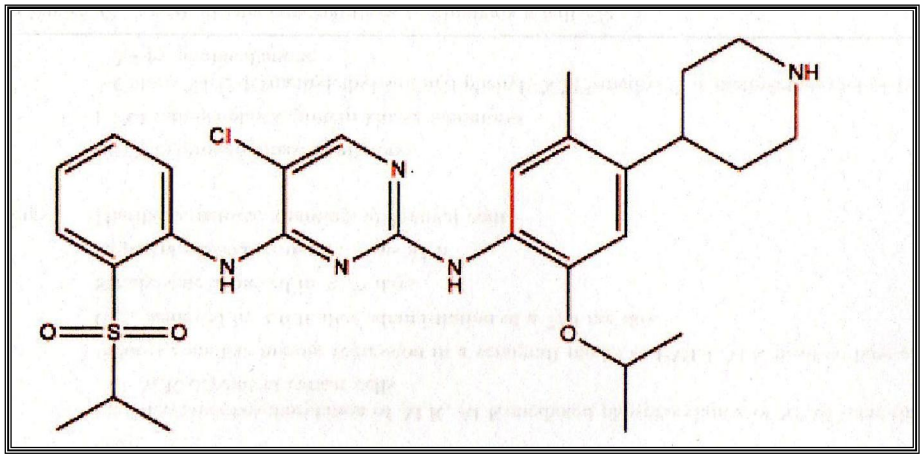


Fig 11.B.1: Chemical structure of ceritinib

The trade names of CRB are LDK378 and zykadia. LDK378 is designed to reduce the possibility of forming reactive metabolites and shows undetectable levels of glutathione (GSH) adducts (<1%) in liver microsomes. LDK378 exhibits low plasma clearance in animals compared to liver blood flow, with the oral bioavailability of above 55% in mouse, rat, dog and monkey. LDK378 show no impact on insulin levels or plasma glucose utilization in the mouse upon chronic dosing up to 100 mg. The recommended dose of zykadia is 750 mg orally daily once

until disease progression toxicity. Zykadia is indicated for the treatment of patients with anaplastic lymphoma kinase (ALK) positive metastatic non-small cell lung cancer (NSCLC).

CRB is a white to almost white or light yellow or light brown powder with p^Ka of 9.7 and 4.1. Zykadia is supplied as printed hard-gelatin capsules containing 150 mg of CRB and the following inactive ingredients: colloidal anhydrous silica, L-hydroxy propyl cellulose, magnesium stearate, micro crystalline cellulose, sodium starch glycolate and hard gelatin capsule shells. The capsule shell is composed of gelatin, indigotine and titanium dioxide. The UV spectrum of CRB is represented in **Fig 11.B.2.**

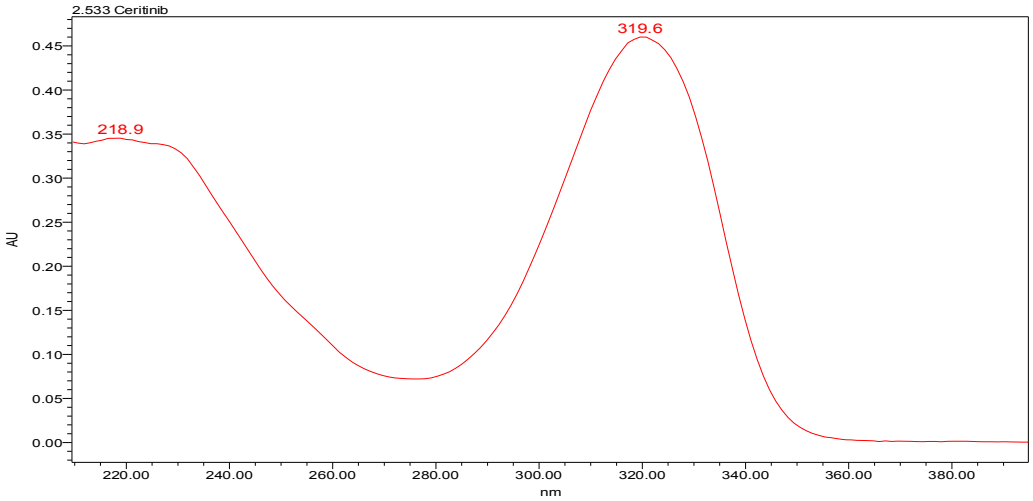


Fig 11.B.2: UV spectrum of ceritinib

Literature Survey: CRB is an anaplastic lymphoma kinase (ALK) inhibitor. Naveen Kumar Ch et al **[190]** reported a novel validated stability indicating RP-HPLC method development for the estimation of ceritinib in its bulk and finished dosage form as per ICH guidelines. Olivier Heudi and co-workers **[191]** developed liquid chromatography-tandem mass spectrometry method for the quantitative analysis of ceritinib in human plasma and its application to pharmacokinetic studies. Alice T Shaw and co-workers **[192]** reported that ceritinib has been approved in the US under 'break through therapy' designation for the second line treatment of ALK-positive non-small cell lung cancer (NSCLC). The recommended dosage of ceritinib is 50 mg administered orally once daily on an empty stomach. Lanshoeft Christian et al **[193]** developed an ultrafast, sensitive, selective and robust LDTD-APCI-MS/MS method

for the quantification of ceritinib in human plasma. Nigel J Waters and co-workers **[194]** reported that ceritinib is a recently approved drug by FDA.

The spectrophotometric methods like UV spectroscopy, mass spectroscopy, conductometry etc., were not reported for ceritinib, till now. This work deals with the UV spectrophotometric method development for the assay of ceritinib from its dosage form (tablets). Hence, the method can be used for routine quality control analysis and also stability. The aim and scope of the proposed work are to develop suitable spectrophotometric method for assay of ceritinib tablet and to perform the validation for the method.

11.B.2 Experimental

11.B.2.1 Instrumentation

The quantitative determination of ceritinib was performed on UV spectrophotometer. Sartorious balance model–TTA225D,ID.No.QC1 017 electronic balance was used for weighing. A shimazdu UV-2450 PC series UV –visible spectrophotometer with slit width 2.0 nm, light source change wave length of 320 nm and wave length range of 200-400nm with sampling interval of 1.2 was used for measuring the absorbance of solutions.

11.B.2.2 Materials and reagents

The reference sample of ceritinib was supplied by M/s Spectrum labs, Hyderabad, Telangana state, India. Commercial formulations of zykadia ^{TM} containing ceritinib was purchased from the local market.

11.B.2.3 Preparation of mobile phase

A mixture of buffer solution and acetonitrile in the ratio of 55:45 %v/v was used as mobile phase.

11.B.2.4 Preparation of buffer solution

Accurately weighed 1.36 grams of sodium dihydrogen orthophosphate was placed in a 1000 mL volumetric flask, added about 900 mL of milli-Q water and degassed to sonicate and finally make up the volume with water and pH was adjusted to 4.5 with dilute ortho phosphoric acid.

11.B.2.5 Preparation of standard solution

Accurately weighed and transferred 10 mg of ceritinib working standards into a 10 mL clean dry volumetric flask, 7 mL of diluents was added, sonicated for 30 minutes and make up to the final volume with diluents to prepare a 1000 µg/mL standard stock solution. From the above stock solution, 1 mL was pipetted out into a 10 mL volumetric flask and then make up to the final volume with diluents. Standard solutions of ceritinib having concentration in the range of 25-150 µg/mL were prepared by diluting stock solution with mobile phase.

11.B.2.6 Preparation of sample solution

Ten tablets of ceritinib sample were weighed and powdered. 10 mg of powdered ceritinib was transferred into 10 mL volumetric flask containing 7 mL of diluents (mixture of methanol and water in 50:50 v/v ratio) and sonicated for 25 minutes, further the volume was made up with diluents and filtered. From the filtered solution, 1 mL solution was pipetted out into a 10 mL volumetric flask and made upto 10 mL with diluents. Sample ceritinib concentration of 100 µg/mL was obtained.

11.B.3.1 UV method development

In the development of the present UV spectrophotometric method for the quantitative determination of ceritinib, the analytical condition were selected after testing the different parameters such as diluents, buffer concentration, and other chromatographic conditions. Our preliminary trials were by using different compositions of diluents consisting of water with buffer and methanol. By using

diluent consisted of methanol - water (50:50, %v/v) best result was obtained and degassed in an ultrasonic bath.

11.B.3.2 Selection of wavelength

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Ceritinib shows λ_{max} at 320 nm. The proposed analytical method is simple, accurate and reproducible.

11.B.4 UV Method Validation

Validation is concerned with assuring that a measurement process produces valid measurements. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

11.B.4.1 Specificity

In the resolution of the analyte peak from the nearest peak, solution of each of the analyte was injected separately and their retention time was noted. The standard working solution containing a mixture of the component being analyze is also injected and each of analyte peaks is check for its resolution from the nearest.

11.B.4.2 Linearity

Six points calibration curve were obtained in a concentration range from 20-150 µg/mL for ceritinib. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.006 x - 0.001 with correlation coefficient 0.9987. The amount of CRB present in the linear solutions and their absorbance at 319.6 nm are given in **Table 11.B.1.**

Table 11.B.1: Amounts of CRB present in the linear solutions

S.No.	Level of the solution	Amount of the drug µg/mL	Absorbance
1	20%	0.2	0.115
2	50%	0.4	0.229
3	70%	0.6	0.375
4	100%	0.8	0.456
5	120%	1.0	0.573
6	150%	1.2	0.688

A graph was plotted between concentration of ceritinib and absorbances, a calibration plot was obtained, which was shown in **Fig 11.B.2.**

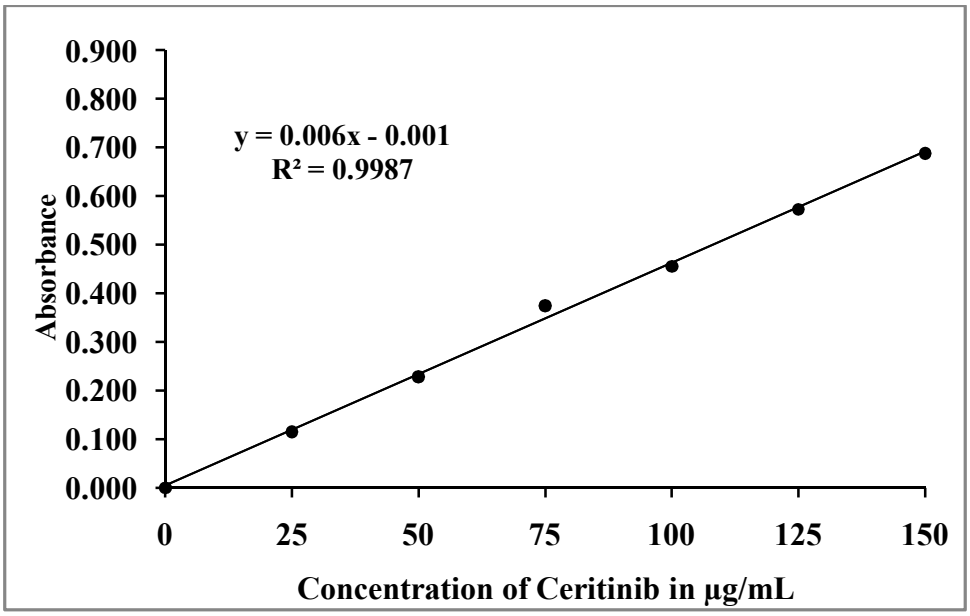


Fig 11.B.2: UV calibration plot of ceritinib

11.B.4.3 Precision

Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeated the analysis six times. Calculate the % assay, mean assay, standard deviation (SD) and % relative standard deviation (%RSD). The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were 0.98% and 0.79%, respectively and the evaluation data of precision study for CRB was shown in **Table 11.B.2.**

Table 11.B.2: Evaluation data of precision study for CRB

Sample No	% Assay	
	Intraday	**Interday**
1	99.79	99.12
2	100.3	98.9
3	100.8	100.4
4	99.8	99.6
5	100.6	98.9
6	100.12	100.73
Mean	100.235	99.6
SD	0.912	0.846
%RSD	0.909	0.849

11.B.4.4 Accuracy

Accuracy of the method is ascertained by standard at three levels. Standard quantity equivalent to 50, 100 and 150% is added in sample. The result revealed that best recoveries of the spiked drug were obtained at each added concentration, indicating that the method was accurate and the evaluation data of accuracy study for CRB was shown in **Table 11.B.3.**

Table 11.B.3: Evaluation data of accuracy study for CRB

% Recovery level	% Recovery	Mean % recovery	SD	%RSD
50	99.8 100.4 99.9	100.03	0.8074	0.8071
100	99.6 100.2 100.9	100.23	0.8294	0.8274
150	99.7 100.6 100.1	100.13	0.8348	0.8337

11.B.4.5 Robustness

The evaluation of robustness should be considered during the development phase. It depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. The result of robustness study of the developed assay method for CRB was established in **Table 11.B.4.** The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory, hence the analytical method would be concluded as robust.

Table 11.B.4: Evaluation data of robustness study for CRB

S.No.	319 nm	320 nm	321 nm
1	0.992	0.995	0.999
2	0.992	0.995	0.999
3	0.993	0.995	0.998
4	0.993	0.996	0.997
5	0.993	0.995	0.998
6	0.994	0.996	0.999
Mean	0.9928	0.9953	0.9983
SD	0.00208	0.00786	0.00798
%RSD	0.21	0.79	0.8

11.B.4.6 System suitability

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

11.B.5 Results and discussion

Ceritinib shows maximum absorbance at 319.6 nm wavelength. In the present UV-Spectrophotometric method, ceritinib obeys Beer`s Law in the concentration range of 20-150 µg/mL. By conducting inter day determinations and intraday determinations under identical conditions the precision of the method is determined. The % RSD values of inter-day and intra-day precision are less than 2, the method is precise. In the linearity studies solutions of 20 to 150% of precision concentration are prepared, their absorbances are measured at 319.6 nm and a graph is drawn between concentration of ceritinib and the absorbances then a linear calibration plot was obtained. The solutions follow Beer's law in the concentration range 20-150 μg/mL and from the slope and standard deviation of the plot LOD and LOQ are calculated and their values are within the limits. The accuracy is evaluated by the addition of standard API`s of ceritinib to pre-analyzed solutions and subsequent recovery studies by the present method. The % recoveries are in the limit 100 ± 1, hence the method is accurate. The ruggedness is determined by the analysis of the sample under different conditions like different analysts / different instruments etc. In the present method ruggedness is determined by conducting the analysis by different analysts under the identical conditions. The % RSD of the ruggedness studies is less than 2, the method is rugged. By using the present UV–spectrophotometric method, the assay of the sample was determined, the % assay of sample is in the limit 100± 1. The present method could be used for the rapid, accurate, precise and economic UV determination of ceritinib in bulk and formulations.

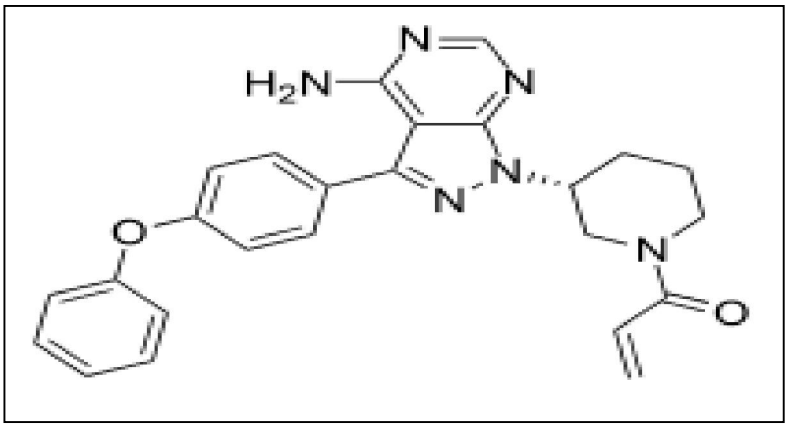
11.B.6 Conclusion

The developed method was completely validated as per ICH guidelines and found to be precise and accurate, as depicted by the statistical data of analysis. High values of correlation co-efficients and small values of intercepts validated the linearity of the calibration plots and obedience to Beer's laws. The RSD values, the slopes and intercepts of the calibration graphs indicate the high reproducibility of the proposed method. The UV-Spectrophotometric method presently developed is a simple, accurate, precise, economic and sensitive for the determination of ceritinib in bulk and dosage forms without any interference from the excipients present. Hence this method could be used for the routine analysis of ceritinib in quality control laboratories.

UV Spectrophotometric Determination of Ibrutinib in Bulk and Dosage Forms

11.C.1 Introduction

Ibrutinib (IBR) is an anticancer drug targeting B-cell malignancies (blood cancer treatment medicines). It was approved by the US FDA (2013). The systematic (IUPAC) name of ibrutinib is given by 1-[(3*R*)-3-[4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo [3, 4-d] pyrimidin-1-yl] piperidin-1-yl] prop-2-en-1-one. The molecular formula and molecular mass of IBR are $C_{25}H_{24}N_6O_2$ and 440.497 grams per mole respectively. The chemical structure of ibrutinib is given in **Fig 11.C.1.**



Literature Survey: IBR is available as imbruvica capsules for oral administration containing 140 mg ibrutinib as the active ingredient. Each capsule also contains the inactive ingredients such as croscarmellose sodium, magnesium stearate, micro crystalline cellulose, sodium lauryl sulfate. The capsule shell contains gelatin, titanium dioxide and black ink. Azvolinsky Ph.D Anna **[195]** reported that IBR is used for the treatment of mantle cell lymphoma and for the treatment of chronic lymphocytic leukaemia. Pan Z et al **[196]** and Honigberg L et al **[197]** reported that IBR is an orally-administered, selective and covalent inhibitor of the enzyme bruton's tyrosine kinase (BTK). Simultaneous quantification of lenalidomide, ibrutinib and its active metabolite PCI-45227 in rat plasma by LC-MS/MS, was developed by Veeraraghavan S and co-workers **[198]**; Sridhar Veeraraghavan and co-workers **[199]** to study pharmacokinetics. Sateesh Thappali and others **[200]** reported that the

ibuprofen and methocarbamol are simultaneously estimated in tablet dosage form in rat plasma by spectrophotometric method.

No other methods were found for the determination of Ibrutinib and its formulations. As there is no UV method in the literature, the author made some investigations to develop a novel UV spectrophotometric method for the assay of Ibrutinib in pure and formulations. The main objectives of the present investigation are, the developed method should be a sensitive, selective, precise, accurate, simple, rapid and economic. The proposed method should be applicable to determine the quality of bulk product and to determine assay of formulations. The UV spectrum of IBR was represented in **Fig 11.C.2.**

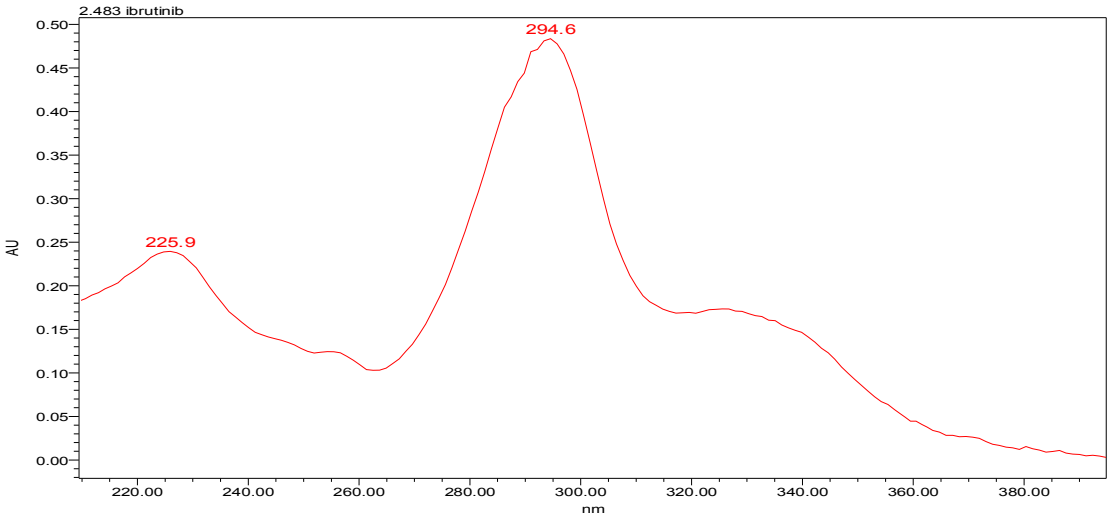


Fig 11.C.2: UV spectrum of ibrutinib

11.C.2 Experimental

11.C.2.1 Instrumentation

Sartorious balance model–TTA225D,ID.No.QC1 017 electronic balance was used for weighing. A shimazdu UV-2450 PC series UV –visible spectrophotometer with slit width 2.0 nm, light source change wave length of 320 nm and wave length range of 200-400nm with sampling interval of 1.2 is used for measuring the absorbance of solutions.

11.C.2.2 Chemicals and reagents

Ibrutinib is a gift sample obtained from M/s Spectrum labs, Hyderabad, Telangana State. All the chemicals used in the validation process are of analytical grade and supplied by Merck Co, Mumbai, India.

11.C.2.3 Preparation of buffer solution

About 1.0 mL of ortho phosphoric acid (OPA) was accurately measured and transferred into a 1000 mL of volumetric flask, added about 900 mL of milli-Q water and degassed to sonicate and finally made up the volume with water.

11.C.2.4 Preparation of mobile phase

0.1% ortho phosphoric acid (OPA) buffer and acetonitrile were mixed together in the ratio 70:30 %v/v by adding 300 mL of acetonitrile solvent to 700 mL of 0.1% OPA buffer solution, sonicated for five minutes and used as mobile phase.

11.C.2.5 Preparation of standard solution (140 μg/mL)

An amount of 140 mg of 99.8% pure API product Ibrutinib was accurately weighed and transferred into a 100 mL clean dry volumetric flask, added 20 mL of diluents (methanol), sonicated for 30 minutes and made up to the final volume with diluents. Then 1.0 mL of above stock solution was transferred into a 10 mL volumetric flask and then made up to the final volume with diluents, and the concentration of resulting solution was 140 µg/mL.

11.C.2.6 Preparation of sample solution

Average weight of five capsules were determined, and one capsule equivalent to 140 mg of ibrutinib was transferred into a 100 mL clean dry volumetric flask, added 20 mL of diluents (methanol), sonicated for 30 minutes and made up to the final volume with diluents. Then the solution was filtered and about 1.0 mL of above

stock solution was transferred into a 10 mL volumetric flask and then made up to the final volume with diluent.

11.C.3 UV method development

In the development of the present UV spectrophotometric method for the quantitative determination of ibrutinib, the analytical conditions were selected after testing the different parameters such diluents, buffer concentration and other chromatographic conditions.

11.C.3.1 Selection of wave length

Scan standard solution of ibrutinib in UV spectrophotometer between 200 - 400 nm on spectrum mode, using diluents as a blank. Ibrutinib shows λ_{max} at 294.6 nm. The proposed analytical method is simple, accurate and reproducible.

11.C.4 UV method validation

The present UV spectrophotometric method is validated as per ICH guidelines for accuracy, precision, linearity, ruggedness and sensitivity. The optical characteristics of ibrutinib were shown in **Table 11.C.1.**

Table 11.C.1: Optical characteristics of ibrutinib

S.No.	Parameter	Value
1	Wave length of absorbance measurements	320 nm
2	Regression equation	Y = 0.006 x
3	Slope (S)	0.006
4	Correlation coefficient (r)	0.9999
5	Beer's law limits	3.5 - 21.0 μg/mL
6	Standard deviation (SD)	25338.1
7	LOD (3.3 σ/S) μg/mL	0.03
8	LOQ (10 σ/S) μg/mL	0.10

11.C.4.1 Recovery studies (Accuracy)

By the addition of standard API of ibrutinib to the pre-analysed sample and the subsequent recovery of the total assay by the present method, the accuracy of the method is determined. To the standard solutions of ibrutinib, 50 , 100 and 150 % of API`s of ibrutinib are added and the resulting solutions are analyzed by the present method. The amounts of ibrutinib present in the solutions recovered were shown in **Table 11.C.2.** %Recovery of ibrutinib is 100± 2, indicating that the method is accurate.

Table 11.C.2: Amounts of ibrutinib recovered

S.No.	% Level of the solution	Amount taken µg/mL	Amount of API added µg/mL	Total amount present µg/mL	Amount recovered	% of recovery
1	50	14	7	21	20.95	99.76
2	100	14	14	28	27.94	99.79
3	150	14	21	35	34.94	99.83

11.C.4.2 Precision

The precision of a method is in agreement with the results obtained when the method is applied to different aliquots of a homogeneous solution. Samples of ibrutinib are analyzed within a day (Intra-day precision) and within different days (Inter-day precision). Intraday precision of IBR was determined by analyzing solutions of 7 μg/mL, 14 μg/mL and 21 μg/mL concentration three times within a day. Inter day precision of IBR was determined by analyzing the samples daily once in a day in three days. The results of precision study for IBR were shown in **Table 11.C.3.**

Table 11.C.3: Results of precision study for IBR

S.No.	% Level	Conc of solution (µg/mL)	Intraday (% RSD)	Inter day (%RSD)
1	50	7	0.075	0.079
2	100	14	0.065	0.058
3	150	21	0.054	0.051

11.C.4.3 Linearity

The linearity of the method for IBR was determined by taking the absorbance of six linear solutions of different concentrations. A calibration plot was constructed between concentration of IBR (µg/mL) and absorbance. In this method, linearity was observed in the concentration range of 3.5 - 21.0 μg/mL with correlation coefficient of 0.9999. The amounts of ibrutinib present in the linear solutions were shown in **Table 11.C.4** and the calibration plot of IBR was shown in **Fig 11.C.3.**

Table 11.C.4: Amounts of ibrutinib present in the linear solutions

S.No.	% Level of the solution	Amount of the drug (µg/mL)	Absorbance
1	25	3.5	0.083
2	50	7.0	0.162
3	75	10.5	0.251
4	100	14.0	0.321
5	125	17.5	0.423
6	150	21.0	0.490

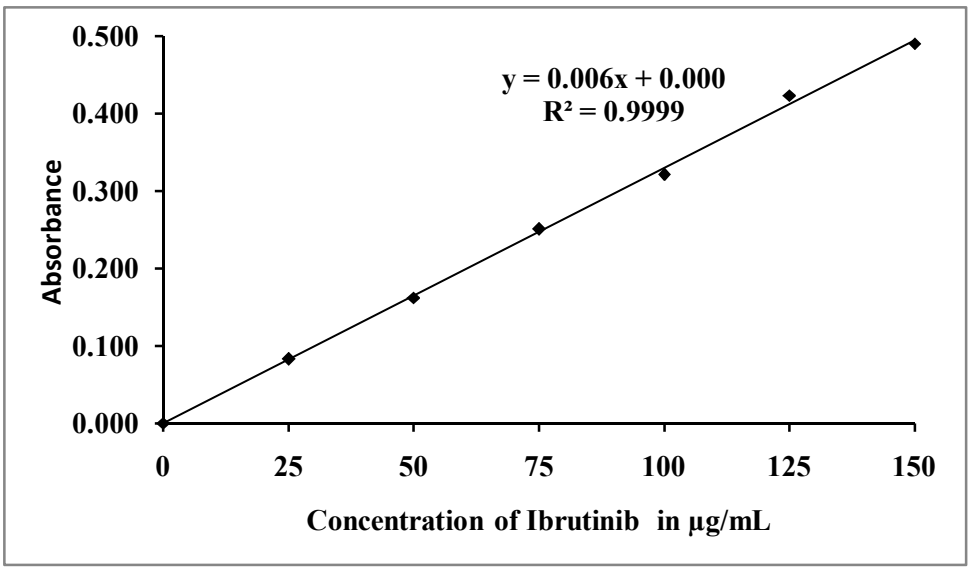


Fig 11.C.3: UV linearity plot of IBR

11.C.4.4 Ruggedness

The ruggedness of an analytical method is determined by the analysis of the same sample of IBR under different conditions by different analysts, by different instruments etc. Presently the ruggedness of the method for IBR was determined by the analysis of the samples of IBR by different analysts under identical conditions. The results of ruggedness of IBR were shown in **Table 11.C.5.**

Table 11.C.5: Results of ruggedness for IBR

S.No.	Amount taken	Analyst – I	%RSD	Analyst – II	% RSD
1	10 µg	9.94 µg	1.334	9.93 µg	1.624

11.C.4.5 Repeatability

Repeatability is determined by the analysis of six solutions of ibrutinib six times. The results of repeatability for IBR were shown in **Table 11.C.6.**

Table 11.C.6: Results of repeatability for IBR*

S.No.	Amount of the drug taken (µg)	Amount found (µg)	% recovery	% RSD
1	10	9.94	99.4	0.082

*Average of six determinations

11.C.4.6 Determination of the assay of the sample imbruvica

Six identical solutions of sample imbruvica were taken and their absorbances were measured. From statistical methods, the % RSD was determined. The result of analysis of the dosage form of imbruvica was shown in **Table 11.C.7.**

Table 11.C.7: Results of analysis of imbruvica in pharmaceutical dosage form

S.No.	Amount of imbruvica taken (mg)	Amount found (mg)	% of assay
1	10	9.94	99.4

11.C.5 Results and discussion

The absorbances of different ibrutinib solutions are taken at 320 nm wave length. Ibrutinib solutions obeys Beer`s Law in the concentration range 3.5 - 21.0 $\mu$g/mL with correlation coefficient of 0.9999. By the present method the sample solutions are analyzed and the % of the drug found is 99.2, which is in the range 100±1. The accuracy of the method is determined by the addition of known amounts of API`S of ibrutinib to the pre-analyzed sample and the total amounts of assay present in the solutions are determined by the present method. The %RSD of the determinations of accuracy are less than 2, hence the method is accurate. From the inter-day and intra-day precision analysis of the ibrutinib solutions under identical conditions, the precision of the method was determined. The % RSD of the precision studies is less than 2, hence the method is precise. The ruggedness is determined by analyzing the samples by different analysts under identical conditions. The % RSD is less than 2, hence the method is rugged. Solutions of ibrutinib are analyzed six times and the repeatability is determined. The % RSD of the repeatability studies is less than 2, and hence the method is repeatable. The LOD and LOQ values were determined, which were in the limits and hence the method is sensitive. Same solutions of the sample imbruvica tablets are analyzed six times, found that the % recovery of the assay of the sample is within the limits 100 ±1. Hence, the method may be used for the determination of ibrutinib in pharmaceutical dosage forms.

11.C.6 Conclusion

In the present study, a simple, precise, accurate and low cost UV-spectrophotometric method is developed for the determination of ibrutinib in bulk and dosage forms. The absorbance of aqueous Ibrutinib solutions are measured by UV-visible spectrophotometer at a wave length of 320 nm. This method obeys Beer's law in the concentration range of 3.5 - 21.0 µg/mL in aqueous media. As per linearity studies, the correlation coefficient, slope and standard deviation are 0.9999, 0.006 and 25338.1 respectively. The % recovery of the assay of the drug in the sample in this method is 99.2 and the method is sensitive in presence of the excipients in the dosage form. According to ICH guidelines, linearity, accuracy, precision, LOD, LOQ were

studied and the method is validated. Hence, the present method is suitable for the accurate, precise, sensitive and low cost determination of Ibrutinib in pure and formulations.

The UV-Spectrophotometric method presently developed is simple, accurate, precise, cost effective and sensitive for the determination of ibrutinib in bulk and dosage forms without any interference from the excipients present. Hence, this method may be used for the routine analysis of ibrutinib in quality control laboratories.