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QUANTITATIVE ESTIMATION OF IBRUTINIB, MIDOSTAURIN IN TABLET DOSAGE FORMS BY RP-HPLC METHOD

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Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Ibrutinib and Midostaurin, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Altima C18 (4.6 x 150mm, 5µm) column using a mixture of ACN, methanol and Phosphate buffer pH4.6 (10:25:65 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 234nm. The retention time of the Midostaurin and Ibrutinib was 2.088, 6.068 ±0.02min respectively. The method produce linear responses in the concentration range of 25-125ppm of Midostaurin and 10-50ppm of Ibrutinib . The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Midostaurin, Ibrutinib, RP-HPLC, validation*

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INTRODUCTION:

Quality can be defined as the character, which defines the grade of excellence. A good quality drug is something, which will meet the established product specifications, can be safely bought and confidently used for the purpose for which it is intended. To get a good quality drug, the manufacturing for making a drug should have quality built into it.

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical chemistry is a sub- Chromatography and its types. Chromatography is a method used for separating organic and inorganic compounds so that they can be analysed and studied. Chromatography is a great physical method for observing mixtures and solvents. The word chromatography means colour separation where chroma means colour and graphy means separation. Chromatography is based on different migration. Solutes with a greater affinity for the mobile phase will spend more time in this phase than solutes that prefer the stationary phase. As the solutes move through the stationary phase thedifferent components are going to be absorbed and are going to stop moving with mobile phase .

Thus, they are separated. This is called as chromatographic development.

The different type of chromatography Adsorption chromatography:

Adsorption chromatography is probably one of the oldest types of chromatography around. Itutilises a mobile liquid or gaseous phase that is absorbed on to the surface of a stationary solid phase. The equilibrium between the mobile and stationary phase accounts for the separation of different solutes.

Partition chromatography:

This form of chromatography is based on thin film formed on the surface of a solid support by a liquid stationary phase .Solutes equilibrates between the mobile phase and the stationaryliquid.

Ion exchange chromatography:

In this type of chromatography, the use of a resin (the stationary solid phase) is used to covalently attach anions or cations to it. Solute ions of the opposite charge in the mobile liquid phase are attracted to the resin by electrostatic forces.

Molecular exclusion chromatography:

Also known as gel permeation or gel filtration, this type of chromatography lacks an attractive interaction between stationary phase and solute. The liquid or gaseous phase passesthrough a porous gel, which separates the molecule according to its size. The pores are normally small and exclude the larger solute molecule, but allow smaller molecule to enter the gel, causing them to flow through a larger volume. This causes the larger molecules to pass through the column at a faster rate than the smaller ones.

Affinity chromatography:

This is the most selective type of chromatography employed. It utilises the specific interaction between one kind of solute molecule and a second molecule that is immobilised on a stationary phase. For example the immobilised molecule may be an antibody to some specific protein. When solute containing a mixture of protein is passed by this molecule, onlythe specific protein is reacted to this antibody, it to the binding stationary phase. This protein is later extracted by changing the ionic strength or PH.

High performance liquid chromatography:

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high –molecular weight poly functional group. HPLC isthe fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in bothdosage forms and biological fluids .In this ,the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3- 10µm.Modern LC uses very small particles for packing.

MATERIALS AND METHODS:

INSTRUMENTS USED

HPLC from WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.

CHEMICALS USED:

Ibrutinib and Midostaurin from Sura Pharma Labs, Water and Methanol for HPLC from LICHROSOLV (MERCK) and Acetonitrile for HPLC from Merck

METHOD VALIDATION

Preparation of standard solution:

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

Further pipette 0.6ml of Ibrutinib and 1ml of Midostaurin from the above 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.

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSIONS:

Optimized Chromatogram (Standard)

Mobile phase : Ьuffer: methanol: ACN (65:25:10v/v) Column : Altima C18 (4.6×150mm, 5.0 µm)

Flow rate : 1 ml/min

Wavelength : 234 nm

Column temp : 35ºC Injection Volume : 10 µl

Run time : 14 minutes

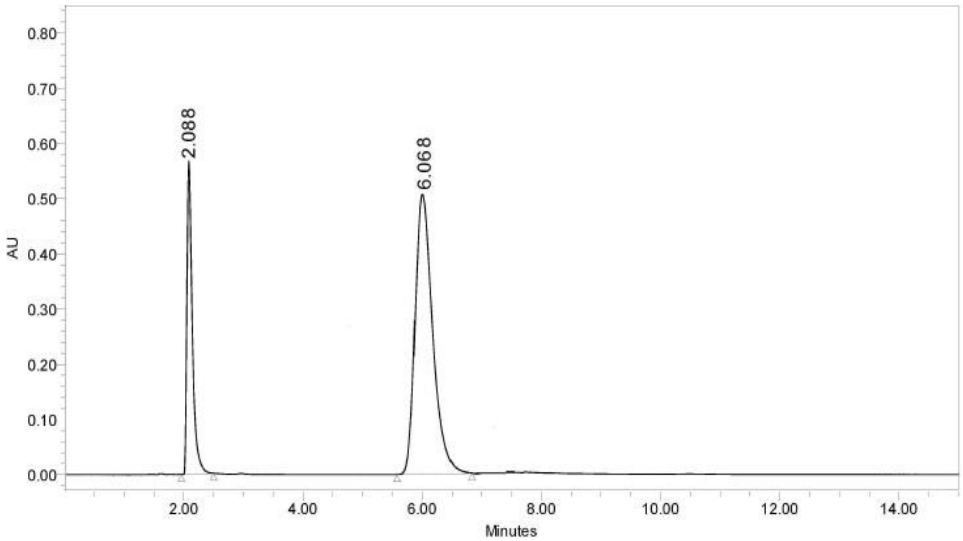

Figure 4.4: Optimized Chromatogram

Table 4.4: Peak results for trail 4

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Midostaurin	2.088	3425414	567934		1.2	5565.6
2	Ibrutinib	6.068	1629855	517734	2.6	1.3	5355.3

Observation:
From the above chromatogram it was observed that the Midostaurin and Ibrutinib peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

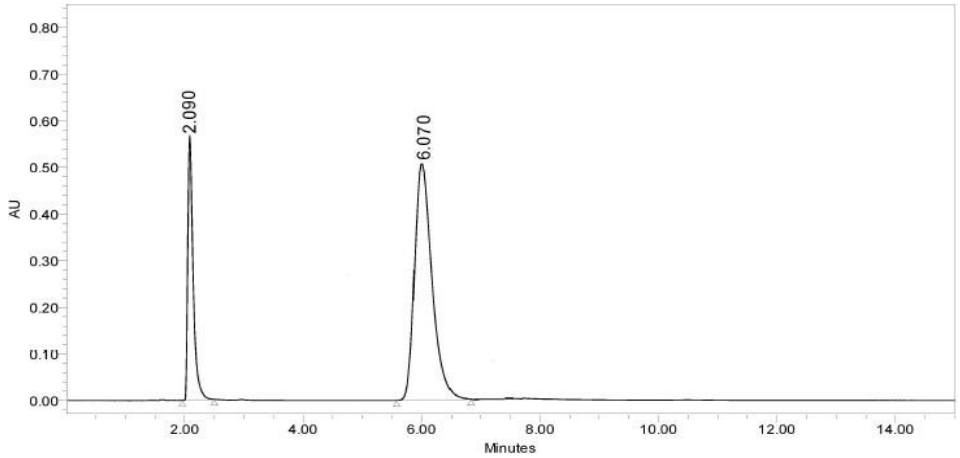

Figure 4.5: Optimized Chromatogram (Sample)

Table 4.5: Optimized Chromatogram (Sample)

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	SP plate count
1	Midostaurin	2.090	3468548	567934		1.0	5565.6
2	Ibrutinib	6.070	16289442	517734	2.6	1.1	5355.3

Acceptance criteria:

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Table 4.6: Results of system suitability for Midostaurin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Midostaurin	2.080	3569413	567918	5569.0	1.0
2	Midostaurin	2.080	3465126	517717	6358.2	1.1
3	Midostaurin	2.080	3598155	567934	5566.5	1.0
4	Midostaurin	2.081	3586492	517732	5354.2	1.1
5	Midostaurin	2.081	3582693	567916	6349.0	1.0
mean			3560376			
Std. Dev			54225.4			
% RSD			1.523025			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4.7: Results of method precession for Ibrutinib:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ibrutinib	2.080	3582265	567918	5567.0	1.0	2.4
2	Ibrutinib	2.080	3586492	517717	5358.2	1.1	2.4
3	Ibrutinib	2.080	3598153	567934	5566.5	1.0	2.4
4	Ibrutinib	2.081	3564126	517732	5354.2	1.1	2.4
5	Ibrutinib	2.081	3569413	562175	5569.0	1.0	2.4
mean			3580090				
Std. Dev			13609.15				
% RSD			0.380134				

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Specificity

The ICH documents define specificity 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 quantitate MIDOSTAURIN and Ibrutinib in drug product.

Assay (Standard):

Table 4.8: Peak results for assay standard

S.No	Name	Rt	Area	igh t	USP Resoluti on	USP Tailin g	USP plate count	ctio n
1	Midostaur in	2.087	3465682	567918		1.0	5569.0	1
2	Ibrutinib	6.067	16235985	517717	2.5	1.1	5358.2	1
3	Midostaur in	2.088	3465414	567934		1.0	5566.5	2
4	Ibrutinib	6.068	16298546	517732	2.5	1.1	5354.2	2
5	Midostaur in	2.088	3465424	567931		1.0	5543.5	3
6	Ibrutinib	6.068	16265212	517735	2.5	1.1	5351.1	3

Assay (Sample):

Table 4.9: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Midostaurin	2.089	3469822	567918		1.0	6569.0	1
2	Ibrutinib	6.069	16259846	517717	2.5	1.1	5358.2	1
3	Midostaurin	2.090	3468548	567934		1.0	5566.5	2
4	Ibrutinib	6.070	16287532	517732	2.5	1.1	5354.2	2
5	Midostaurin	2.090	3468144	567811		1.0	5392.1	3
6	Ibrutinib	6.070	16282432	517625	2.5	1.1	5565.0	3

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

The % purity of Midostaurin and Ibrutinib in pharmaceutical dosage form was found to be 99.6%.

Table 4.5: Optimized Chromatogram (Sample)

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Midostaurin	2.090	3468548	567934	--	1.0	5565.6
2	Ibrutinib	6.070	16289442	517734	2.6	1.1	5355.3

Linearity

Chromatographic Data for Linearity Study: Midostaurin:

Table 4.10: Chromatographic Data for Linearity Study

oncentration Level (%)	Concentration g/ml	Average Peak Area
33.3	25	1010253
66.6	50	2049375
100	75	3072707
133.3	100	3921069
166.6	125	4952814

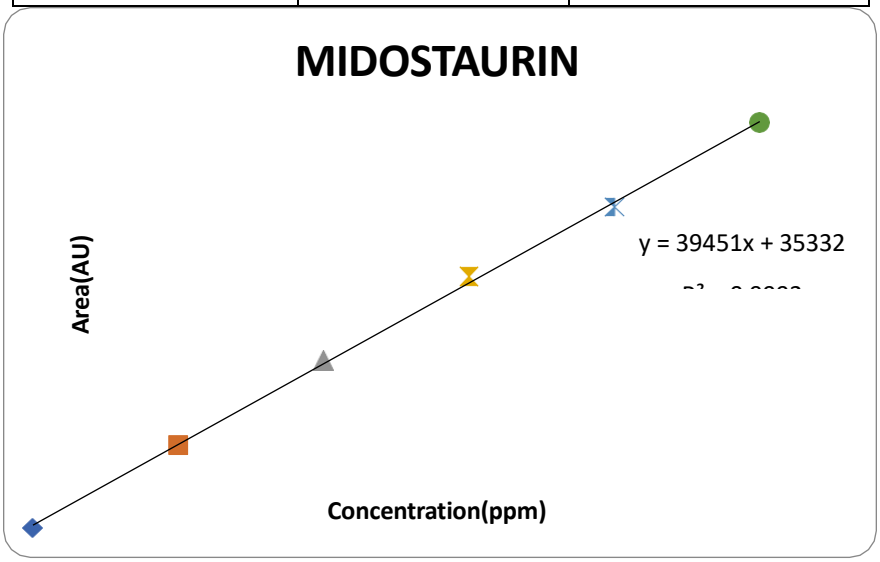

Figure 4.23: Calibration graph for Midostaurin

Linearity plot:

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

Y = mx + c

Slope (m) = 39451 Intercept (c) = 35332
Correlation Coefficient (r) = 0.999

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

Conclusion:
Correlation Coefficient (r) is 0.99, and the intercept is 35332. These values meet the validation criteria.

Ibrutinib

Table 4.11: Ibrutinib Values

oncentration Level (%)	Concentration g/ml	Average Peak Area
33	10	8040806
66	20	14318418
100	30	21087986
133	40	27913927
166	50	34584742

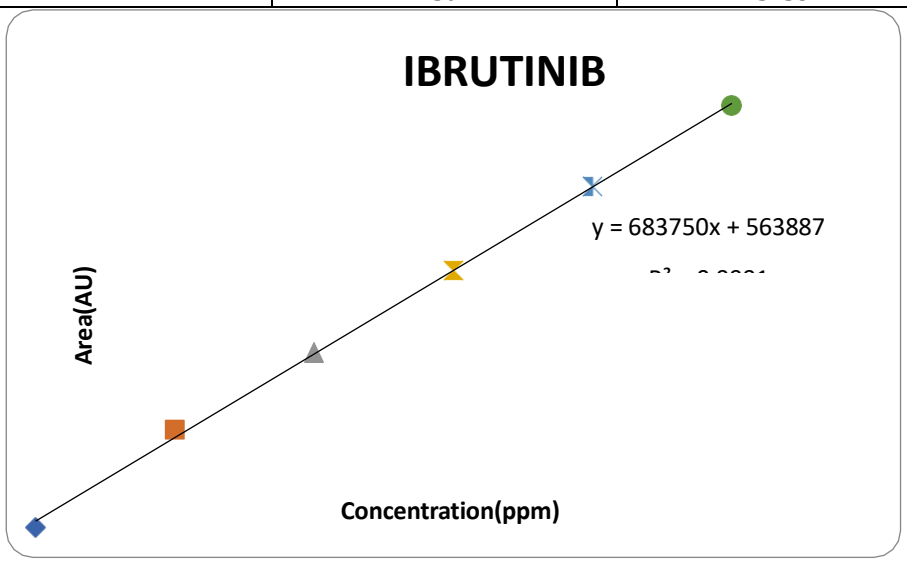

Figure 4.24: Calibration graph for Ibrutinib

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) = 68375 Intercept (c) = 56388
Correlation Coefficient (r) = 0.999

Validation criteria:

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion:
Correlation Coefficient (r) is 0.99, and the intercept is 56388. These values meet the validation criteria.

Precision:
The precision 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.

Repeatability
Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

4.12: Results of repeatability for Midostaurin:

Sno	Name	Rt	Area	Height	SP plate count	USP Tailing
1	Midostaurin	2.084	3569413	567918	5569.0	1.0
2	Midostaurin	2.083	3465126	517717	5358.2	1.1
3	Midostaurin	2.082	3598153	567934	5566.5	1.0
4	Midostaurin	2.081	3586492	517732	5354.2	1.1
5	Midostaurin	2.080	3582695	567918	5568.0	1.0
mean			3560376			
Std. Dev			54225.26			
% RSD			1.523021			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 4.13: Results of method precision for Ibrutinib:

Sno	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP Tailing**	**USP Resolution**
1	Ibrutinib	6.056	1582265	567918	5569.0	1.0	2.6
2	Ibrutinib	6.057	1586492	517718	5358.2	1.1	2.6
3	Ibrutinib	6.058	1598153	567934	5566.5	1.0	2.6
4	Ibrutinib	6.059	1564126	517732	5354.2	1.1	2.6
5	Ibrutinib	6.060	1569413	562175	5569.0	1.0	2.6
mean			1580090				
Std. Dev			13609.15				
% RSD			0.86129				

Acceptance criteria:

- %RSD for sample should be NMT 2

- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Day 1:

Table 4.14: Results of Intermediate precision for MIDOSTAURIN

S.No	Name	Rt	Area	Height	SP plate count	USP Tailing
1	Midostaurin	2.081	3481578	567918	5569.0	1.0
2	Midostaurin	2.082	3458122	517718	5358.2	1.1
3	Midostaurin	2.083	3426582	567934	5566.5	1.0
4	Midostaurin	2.084	3465713	517732	5354.2	1.1
5	Midostaurin	2.085	3451475	567918	5567.0	1.0
6	Midostaurin	2.085	3452107	567515	5358.2	1.1
mean			3455928			
Std. Dev			18188.93			
% RSD			0.5			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2

Table 4.15: Results of Intermediate precision for Ibrutinib

S.No	Name	Rt	Area	Height	SP plate count	USP Tailing	USP Resolution
1	Ibrutinib	6.061	15481578	567918	5569.0	1.0	2.5
2	Ibrutinib	6.062	15369853	517717	5356.2	1.1	2.5
3	Ibrutinib	6.063	15248455	567934	5561.5	1.0	2.5
4	Ibrutinib	6.064	15874693	517735	5357.2	1.1	2.5
5	Ibrutinib	6.064	15236548	567932	5562.0	1.0	2.5
6	Ibrutinib	6.064	15217546	567131	5358.2	1.1	2.5
mean			15404778				
Std. Dev			251288.4				
% RSD			1.7				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

DAY 2

Table 4.16: Results of Intermediate precision Day 2 for MIDOSTAURIN

S.No	Name	Rt	Area	Height	SP plate count	USP Tailing
1	Midostaurin	2.081	3481578	567918	5567.0	1.0
2	Midostaurin	2.082	3458122	517717	5359.3	1.1
3	Midostaurin	2.083	3426580	567934	5565.4	1.0
4	Midostaurin	2.084	3465713	517732	5355.3	1.1
5	Midostaurin	2.085	3451477	567918	5568.1	1.0
6	Midostaurin	2.085	3452109	567515	5359.3	1.1
mean			3455928			
Std. Dev			18188.93			
% RSD			0.5			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2

Table 4.17: Results of Intermediate precision for Ibrutinib

S.No	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP Tailing**	**USP Resolution**
1	Ibrutinib	6.061	15481578	567918	5568.0	1.0	2.5
2	Ibrutinib	6.062	15369853	517718	5359.3	1.1	2.5
3	Ibrutinib	6.063	15248455	567934	5565.6	1.0	2.5
4	Ibrutinib	6.064	15874693	517732	5355.3	1.1	2.5
5	Ibrutinib	6.064	15236548	567935	5568.1	1.0	2.5
6	Ibrutinib	6.064	15217546	567132	5359.3	1.1	2.5
mean			15404778				
Std. Dev			251289.3				
% RSD			1.7				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:
Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 4.21: Accuracy results for Midostaurin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	mean Recovery
50%	1543792	37.4	37.53	102.9	
100%	3035881	76	75.2	101.3	
150%	4451006	112.4	112.48	98.4	100.8%

Table 4.22: Accuracy results for Ibrutinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	mean Recovery
50%	1084421	15	15.06	101.2	
100%	2096068	30	29.7	99.5	99.7%
150%	3112685	45	44.9	99.6	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit of detection
The detection limit 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

Table 4.23: Midostaurin response standard deviation

Concentration g/ml	Average Peak Area
25	1010253
50	2049375
75	3072707
100	3921069
125	4952814

σ = 58777.45

S= 39451

Table 4.24: Ibrutinib response standard deviation

Concentration g/ml	Average Peak Area
10	8040808
20	14318416
30	21087984
40	27913929
50	34584742

σ = 176374

S= 68375

Result:

Midostaurin:

=3.3 × 58777.45/39451

=4.9µg/ml

Ibrutinib :
=3.3 × 176374/68375
=8.5µg/ml

Limit of quantitation

The quantitation limit 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:

Midostaurin:

=10×58777.45/39451

= 14.8µg/ml

Ibrutinib :

=10 × 176374/68375

= 25.7µg/ml

Robustness

The robustness 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 Midostaurin and Ibrutinib . The method is robust only in less flow condition and the method is robust even by change in the mobile phase ±5%. The standard samples of Midostaurin and Ibrutinib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

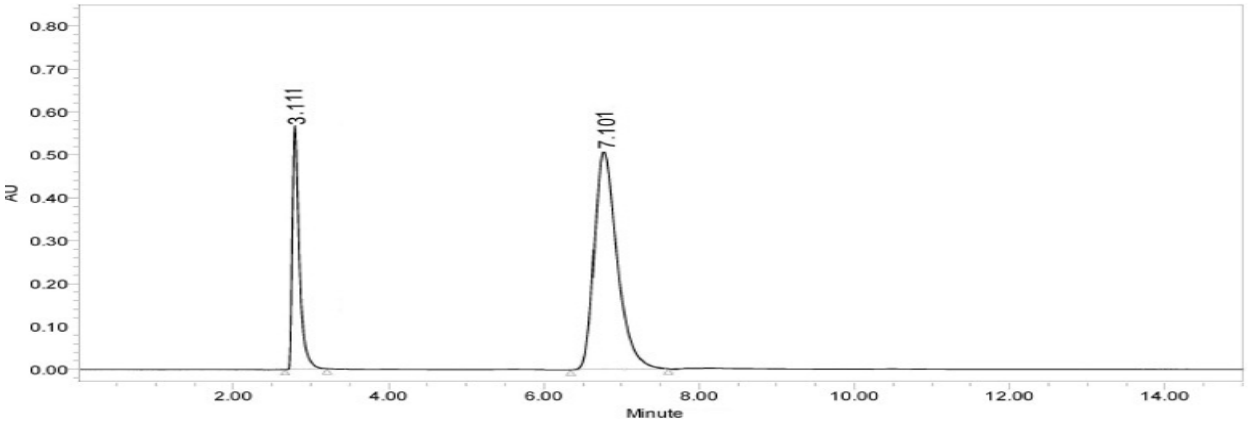

Figure 4.51: Chromatogram showing less flow of 0.9ml/min

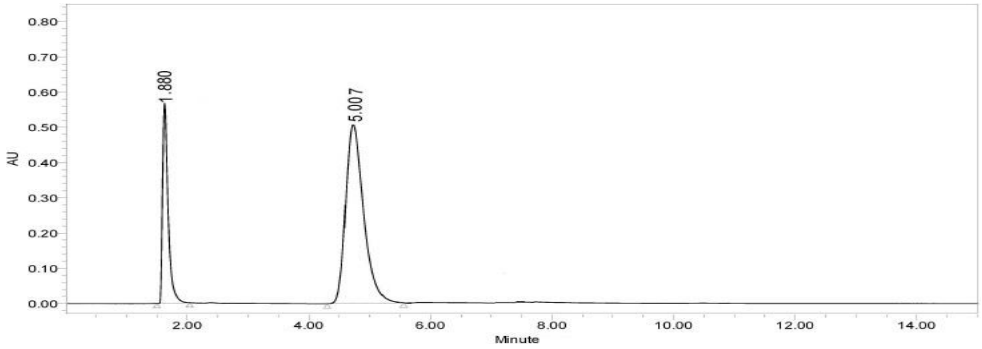

Figure 4.52: Chromatogram showing more flow of 1.1 ml/min Variation of mobile phase organic composition

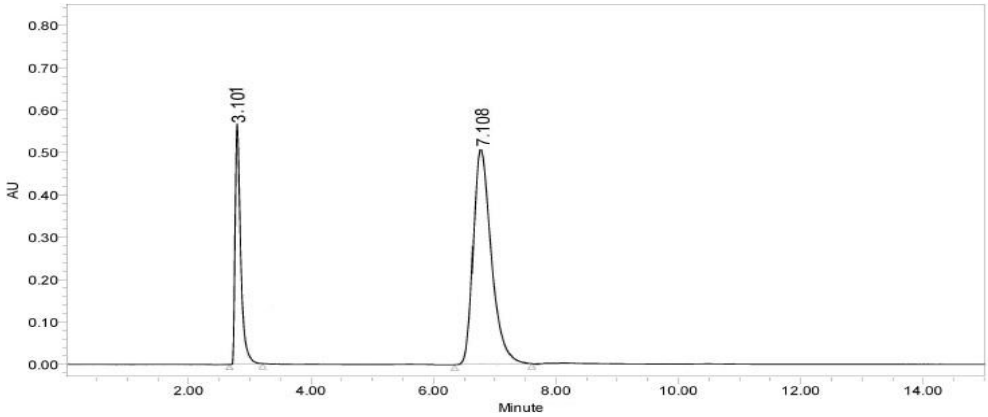

Figure 4.53: Chromatogram showing less organic composition

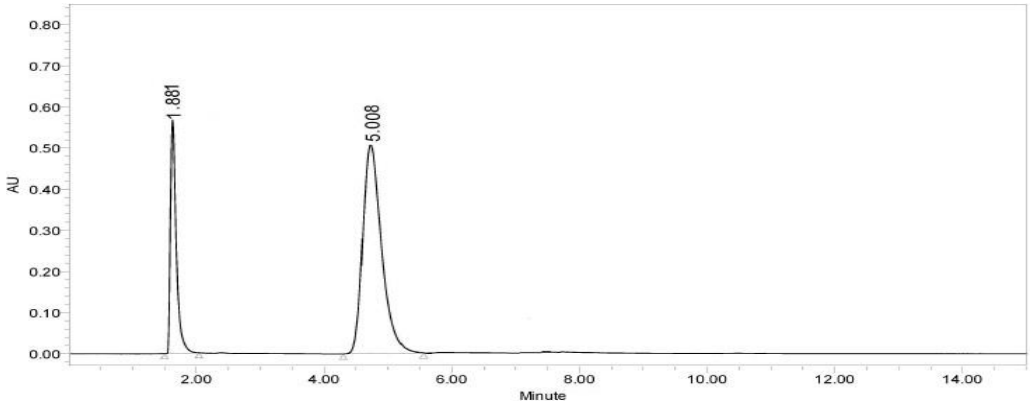

Figure 4.54: Chromatogram showing more organic composition

Midostaurin:

Table 4.25: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	3425412	2.088	5568.3	1.0
Flow rate of 0.9 mL/min	3425283	3.111	5922.1	1.2
Flow rate of 1.1 mL/min	3517878	1.880	5868.9	1.2
Less aqueous phase	3175486	3.101	5836.3	1.2
more aqueous phase	3365432	1.881	5282.7	1.1

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Ibrutinib:

Table 4.26: Sam Analysis Values

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029853	6.068	5359.3	1.1
Flow rate of 0.9 mL/min	1738318	7.101	5999.2	1.2
Flow rate of 1.1 mL/min	1638305	5.007	5989.1	1.1
Less aqueous phase	1973723	7.108	5387.4	1.1
More aqueous phase	2102839	5.008	5938.2	1.1

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 234nm 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 Altima C18 because it was giving good peak. 35ºC 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 ACN, methanol and Phosphate buffer pH4.6 (10:25:65 v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 14min because analyze gave peak around 2.088, 6.068 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 25-125ppm of Midostaurin and 10-50ppm of Ibrutinib of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. In the present investigation, a simple, sensitive, precise and accurate RP- HPLC method was developed for the quantitative estimation of Ibrutinib and Midostaurin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Ibrutinib and Midostaurin was freely soluble in ethanol, methanol and sparingly soluble in water. ACN, methanol and Phosphate buffer pH4.6 (10:25:65 v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Ibrutinib and Midostaurin in bulk drug and in Pharmaceutical dosage forms.

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