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Quantitative Estimation of Ibrutnib, Midostaurin in Tablet Dosage Forms by RP HPLC Method

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A B S T R A C T

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.

INTRODUCTION

Ibrutinib is an anti-cancer drug used for the treatment of mantle cell lymphoma. Mantle cell lymphoma is a fast growing cancer which initiates from the cells of immune system. It blocks the abnormal protein that signals cancer cells multiplication and finally stops dispersion of cancer. Ibrutinib is a very small molecule and acts by binding to the protein permanently. Very less analytical methods are available in the literature which include HPLC, UPLC10, LC-MS/MS methods in rat plasma, human plasma and mouse plama. In this paper a new method was proposed for the quantification of Ibrutinib[1].

Midostaurin is chemically N-((9S,10R,11R,13R)-10-methoxy-9-methyl-1-oxo-2, 3, 10, 11, 12, 13-hexahydro-9,13-epoxy-1H,9H-diindolo(1,2,3-GH:3',2',1'-lm) pyrrolo(3,4j) (1,7) benzodiazonin-11-yl)-n-methylbenzamide.[1] It has a molecular formula of C35H30N4O4 and molecular weight of 570.649 g/mol. Midostaurin is soluble in dimethyl sulfoxide and insoluble in water. It has pKa value of 13.45 (strongly acidic) and -0.83 (strongly basic). Partition coefficient of Midostaurin is 5.81.Water solubility of Midostaurin is 0.0157 mg/ml. The solubility in dimethyl sulfoxide is 14mg/ml. It is a semi-synthetic derivative of staurosporine, an alkaloid from the bacterium Streptomyces staurospores. Midostaurin is a multikinase inhibitor being developed by Novartis Pharmaceuticals[2]. In April 2017, Midostaurin was approved by Food and drug administration (FDA). It acts as a tyrosine kinase and multi-targeted protein kinase inhibitor for the treatment of myeloid leukemia, myelodysplastic syndrome and systemic mastocytosis. Midostaurin is a synthetic indolocarbazole multikinase inhibitor with potential antiangiogenic and antineoplastic activities. Midostaurin inhibits PKC-alpha, VEGFR-2, PDGFR and FLT-3 kinases, which may result in disruption of the cell cycle, inhibition of proliferation, apoptosis, and inhibition of angiogenesis in susceptible tumors. From the literature survey, it was found that few chromatographic methods were developed for the estimation of Midostaurin in bulk and pharmaceutical preparations[3-5]. Hence there was need to develop a new, simple, rapid, precise and accurate reverse phase chromatographic methods to estimate Midostaurin in capsule dosage form. The proposed method was optimized and validated according to International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Materials

Ibrutinib and Midostaurin were procured from Sura labs, Telangana. Water and Methanol for HPLC was procured from LICHROSOLV (MERCK). Acetonitrile for HPLC was purchased from Merck.

Instrumentation

Chromatographic conditions were developed for the analytical technique using Waters Alliance 2695 HPLC with PDA Detector 996 model. The column was Symmetry ODS C18 with dimension 4.6mm×150mm length and particle size packing 5µm.

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.

System Suitability

Accurately weigh and transfer 10 mg of Ibrutinib and Midostaurin 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 out 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 Diluent.

Linearity

Accurately weigh and transfer 10 mg of Ibrutinib and Midostaurin 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). Solutions were prepared containing 20ppm, 40ppm, 60ppm, 80ppm, 100ppm, concentrations of Ibrutinib and 60ppm, 80ppm, 100ppm, 120ppm, 140ppm, concentrations of Midostaurin. Inject each level into the chromatographic system and measure the peak area.

Precision

Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of An analytical method is usually expressed as the standard deviation correlative standard deviation (coefficient of variation) of series of measurements.

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150 % of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For 50%, 150% concentration five sets and for 100% three sets were prepared and injected.

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), variation of mobile phase i.e. Methanol: 0.1% Orthophosphoric acid (64:36% v/v) was taken in the ratio and 69:31, 59:41 instead of 64:36 remaining conditions are same.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae LOD=3.3*σ/slope, LOQ=10*σ/slope (Where σ=the standard deviation of the response and S= Slope of calibration curve).

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of Ibrutinib and Midostaurin. The mobile phase containing ƅuffer: ɱethanol: ACN (65:25:10v/v) was found ideal to resolve the peak of Ibrutinib and Midostaurin. Retention time of Ibrutinib and Midostaurin were 2.090 and 6.070 min respectively. System suitability parameters were evaluated and results shown in (Table-2), which were within acceptance criteria. Result of assay is shown in Table3. Results of intraday and interday precision were shown in the (Table-4&5). LOD and LOQ values were placed in Table-6. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase. The result obtained implies method is robust for routine qualitative analysis (Table-7).

Table 1 - Observations of sample Chromatogram.

S.No	Naɱe	Retention tiɱe(ɱin)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Midostaurin	2.090	3468547	567933		1.0	5565.5
2	Ibrutinib	6.070	16289441	517733	2.5	1.1	5355.2

Table 2a-: Results of system suitability parameters for Midostaurin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Midostaurin	2.080	3569412	567917	5568.0	1.0
2	Midostaurin	2.080	3465125	517719	6359.2	1.1
3	Midostaurin	2.080	3598154	567933	5565.5	1.0
4	Midostaurin	2.081	3586491	517733	5355.2	1.1
5	Midostaurin	2.081	3582694	567917	6348.0	1.0
mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Table 2b-: Results of system suitability parameters for Ibrutinib

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ibrutinib	2.080	3582264	567917	5568.0	1.0	2.5
2	Ibrutinib	2.080	3586491	517719	5359.2	1.1	2.5
3	Ibrutinib	2.080	3598154	567933	5565.5	1.0	2.5
4	Ibrutinib	2.081	3564125	517733	5355.2	1.1	2.5
5	Ibrutinib	2.081	3569412	562173	5568.0	1.0	2.5
mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

Table 3-: Results of Assay

S.No.	**Name of Compound**	**% Purity**
1	Midostaurin	99.6%
2	Ibrutinib	99.2%

Table 4 -: Results of Intermediate precision for Midostaurin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Midostaurin	2.081	3481579	567917	5568.0	1.0
2	Midostaurin	2.082	3458121	517719	5359.2	1.1
3	Midostaurin	2.083	3426581	567933	5565.5	1.0
4	Midostaurin	2.084	3465712	517733	5355.2	1.1
5	Midostaurin	2.085	3451476	567917	5568.0	1.0
6	Midostaurin	2.085	3452106	567514	5359.2	1.1
mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Table 4a-: Results of Intermediate precision for Ibrutinib

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ibrutinib	6.061	15481579	567917	5568.0	1.0	2.5
2	Ibrutinib	6.062	15369852	517719	5359.2	1.1	2.5
3	Ibrutinib	6.063	15248454	567933	5565.5	1.0	2.5
4	Ibrutinib	6.064	15874692	517733	5355.2	1.1	2.5
5	Ibrutinib	6.064	15236547	567933	5568.0	1.0	2.5
6	Ibrutinib	6.064	15217547	567133	5359.2	1.1	2.5
mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

Table 5-: Results of method precision for Ibrutinib

Sno	Namȩe	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ibrutinib	6.056	1582264	567917	5568.0	1.0	2.5
2	Ibrutinib	6.057	1586491	517719	5359.2	1.1	2.5
3	Ibrutinib	6.058	1598154	567933	5565.5	1.0	2.5
4	Ibrutinib	6.059	1564125	517733	5355.2	1.1	2.5
5	Ibrutinib	6.060	1569412	562173	5568.0	1.0	2.5
ɱean			1580089				
Std. Dev			13609.81				
% RSD			0.861332				

Table 5a-: Results of method precision for Midostaurin

Sno	Namȩe	Rt	Area	Height	USP plate count	USP Tailing
1	Midostaurin	2.084	3569412	567917	5568.0	1.0
2	Midostaurin	2.083	3465125	517719	5359.2	1.1
3	Midostaurin	2.082	3598154	567933	5565.5	1.0
4	Midostaurin	2.081	3586491	517733	5355.2	1.1
5	Midostaurin	2.080	3582694	567917	5568.0	1.0
ɱean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Table 6: LOD and LOQ

S.No.	**Name of Compound**	**LOD (µg/ml)**	**LOQ (µg/ml)**
1	Midostaurin	4.9	14.8
2	Ibrutinib	8.5	25.7

Table 7a-: Robustness –System suitability results for Midostaurin

Paraɱeter used for saɱple analysis	**Peak Area**	**Retention Tiɱe**	**Theoretical plates**	**Tailing factor**
Flow rate of 1.0 ɱL/ɱin	3425413	2.088	5568.2	1.0
Flow rate of 0.9 ɱL/ɱin	3425282	3.111	5922.2	1.2
Flow rate of 1.1 ɱL/ɱin	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
ɱore aqueous phase	3365431	1.881	5282.6	1.1

Table 7b-: Robustness System suitability results for Ibrutinib

Paraɱeter used for saɱple analysis	**Peak Area**	**Retention**	**Theoretical plates**	**Tailing factor**
Flow rate of 1.0 ɱL/ɱin	2029854	6.068	5359.2	1.1
Flow rate of 0.9 ɱL/ɱin	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
ɱore aqueous phase	2102838	5.008	5938.1	1.1

DISCUSSION AND 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. Hence the suggested RP-HPLC method can be used for routine analysis of Ibrutinib and Midostaurin in API and Pharmaceutical dosage form. The proposed RP-HPLC method was used for the simultaneous estimation of Ibrutinib and Midostaurin was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing Ibrutinib and Midostaurin.

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