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DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR QUANTITATIVE ESTIMATION OF MIDOSTAURIN IN CAPSULE DOSAGE FORM

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ABSTRACT

A novel, simple, accurate, precise and reproducible RP-HPLC method was developed and avalidated for estimation of Midostaurin in bulk and capsule dosage form. Objective was under optimized chromatographic conditions on Thermo Scientific C18 Column (250 mm × 4.6 mm id, 5 µ) using mobile phase composed of water, acetonitrile and trifluroacetic acid in the ratio of (20:80: 0.1%v/v). The separation was achieved using an isocratic method with flow rate 1ml/min at 30^0C. The effluent was monitored at 293 nm. The retention time was found to be 5.40 min. The method was linear over concentration range of 10-80 µg/ml with regression coefficient 0.9994. The developed method was validated as per ICH guidelines.

KEYWORDS: Midostaurin, Validation, HPLC.

ABBREVIATIONS

PKC alpha: Protein kinase C alpha; VEGFR-2: Vascular Endothelial Growth Factor Receptor- 2; PDGFR: Platelet-derived growth factor receptor; FLT-3: fms like tyrosine-3.

INTRODUCTION

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 $C_{35}H_{30}N_4O_4$ 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. [2] It is a semi-synthetic derivative of staurosporine, an alkaloid from the bacterium *Streptomyces staurosporeus*.[3] Midostaurin is a multikinase inhibitor being developed by Novartis Pharmaceuticals. In April 2017, Midostaurin was approved by Food and drug administration (FDA).[4]

It acts as a tyrosine kinase and multi-targeted protein kinase inhibitor for the treatment of myeloid leukemia, myelodysplastic syndrome and systemic mastocytosis.[5] 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.[6]

From the literature survey, it was found that few chromatographic methods were developed for the estimation of Midostaurin in bulk and pharmaceutical preparations.[7,8] 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 validatedaccording to International Conference on Harmonization (ICH) guidelines.[9]

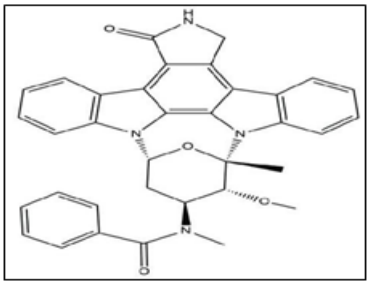

Fig 1: Structure of Midostaurin.

MATERIALS AND METHODOLOGY

Chemicals and Reagents

An analytically pure Midostaurin working standard was procured from Central Drugs Testing Laboratory, Mumbai with defined potency (99.9% as is basis). TAURITMO (25mg) Midostaurinsoft gelatincapsuleswere received as a gift sample from Assistant Drug Controller Office, Air Cargo, and Mumbai.

Acetonitrile, HPLC Grade from Rankem, Trifluroacetic acid AR Grade from Spectrochemand water -milli-Q Grade were used during the analysis.

Instrument

Thermo scientific Dionex Ultimate 3000 was a connected to a computer. Thermo Scientific Dionex Chromelon HPLC using software data system version 7.2.6 with LC instrument control was used for chromatography.

Determination of wavelength

About 10.0 mg of working Midostaurin was transferred to the 20 ml volumetric flask(500ug/ml) and the volume was made up to the mark with diluent{Mixture of Acetonitrile and water (50:50)}. The aliquot portions of standard stock solutions of Midostaurin were diluted appropriately with diluent to obtain concentration of 50µg/ml of drug. The solution were scanned in the range of 200-400nm.The average absorbance maximum of Midostaurin was found at 293nm. As a result 293nm was chosen as final wavelength for the method development and validation.

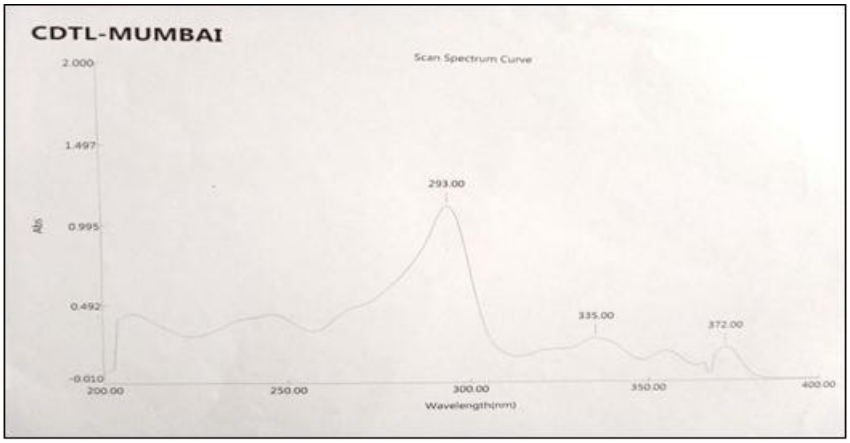

Fig 2: Midostaurin UV Spectrum.

Optimized chromatographic conditions

The chromatographic separation was carried out on Syncronis C18 column (250mm x 4.6mm, 5µm) at 30c with a mixture of water, Trifluroacetic acid (0.1%) and acetonitrile in the ratio of 20:80.0.1%v/v contain as mobile phase. The detection was carried out at 293nm, 10µl injection volume was selected with the flow rate 1ml/min.The retention times was found to be 5.40 minutes.

Preparation of Mobile phase

The mixture of 25mM water, acetonitrile and TFA in the ratio of (20;80:0.1)v/v. it was then sonicated using ultrasonic bath for 10 minutes and was filtered using 0.2 u nylon filter.

Diluent Preparation

On the basis of Molecular structure and chemical nature of Midostaurin, Mixture of Acetonitrile and water (50:50) was selected as diluent for preparation of standard and sample preparation.

Preparation of standard solution

Accurately weighed about 10mg of Midiostaurin standard was transferred in a 20ml volumetric flask and dissolved by sonication in sufficient diluent then volume made with diluent (500ug/ml). Then 2ml from above stock solution was diluted up to 20ml with same diluent (50ug/ml) which is treated as 100% target concentration.

Preparation of sample solution

Ten capsules of TAURITMO (25mg) were weighed and average weight was calculated. Weight equivalent to 25 mg of Midostaurin was poured in 50ml of volumetric flask, 20 ml of diluent was added and sonicated for 15 minutes. Final dilution was made up to 50ml with diluent (500ug/ml).Then 1ml from above stock solution was diluted up to 10ml with the same diluent (50ug/ml).

VALIDATION OF DEVELOPED METHOD

The method developed was validated as per ICH guidelines for linearity, accuracy, precision, robustness and specificity.

System suitability

It is an integral part of method development. A blank preparation (single injection) and standard preparation (six replicate) at the working concentration (50ug/ml) were injected into

the HPLC and the chromatograms were recorded to evaluate the parameters like area, retention time,number of theoretical plates and tailing factors.

Table no. 3: System suitability.

SYSTEM SUITABILITY -				
SR.NO.	**Area**	**Retention time**	**Tailing factor**	**Theoretical Plates**
1	53957	5.42	1.37	15399
2	53937	5.47	1.35	15661
3	53964	5.47	1.35	15758
4	53977	5.47	1.34	15723
5	53958	5.46	1.35	15632
6	53824	5.38	1.34	15591
MEAN	53936	5.445	1.35	15627.3
SD	56.45	0.0372	0.0109	127.15
%RSD	0.1046	0.6847	0.8114	0.813
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NLT 2000

Specificity

It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained. For specificity blank, standard drug solution (50ug/ml) and sample solution (50ug/ml) were injected into the HPLC and their chromatogram was recorded. It reveal that the peaks obtained in the standard and sample solution at working concentration are only because of drugs ,as blank have no peak at the retention time of Midostaurin. Accordingly it can be concluded that, the method issaid to be specific.

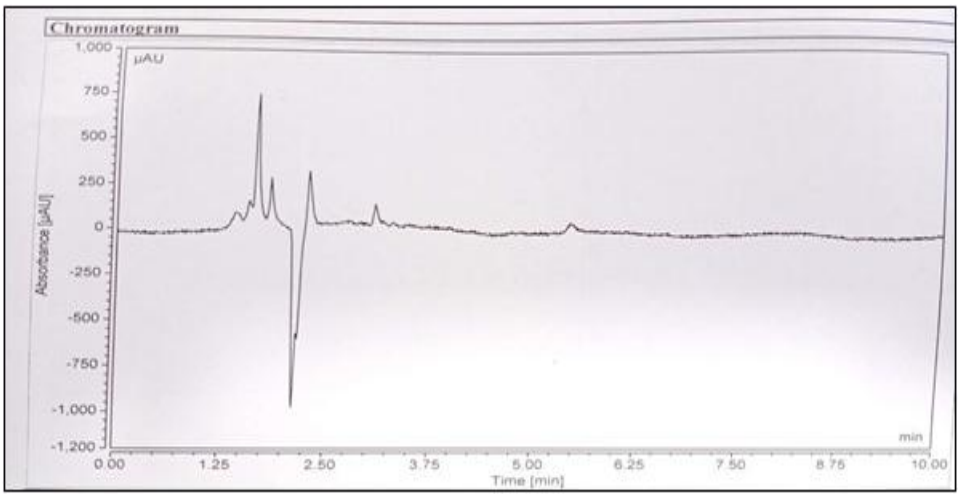

Fig 4: Chromatogram of Blank Solution.

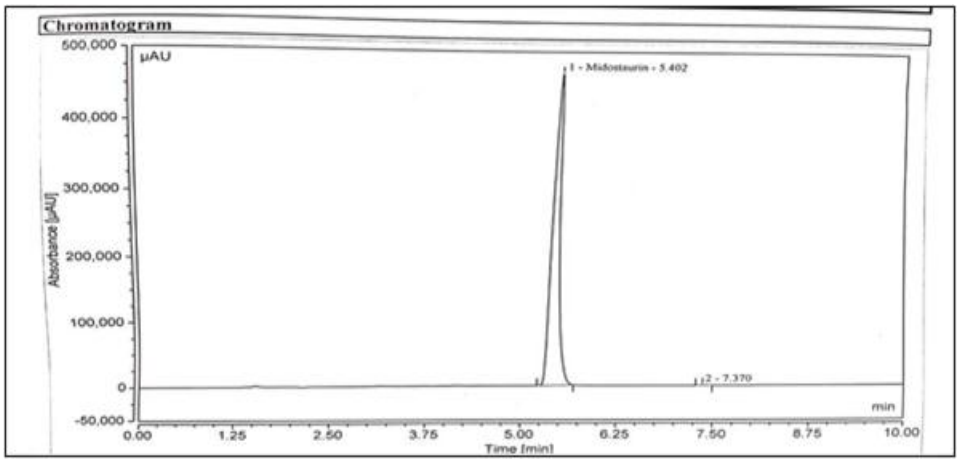

Fig 5: Chromatogram of Midostaurin Standard Solution.

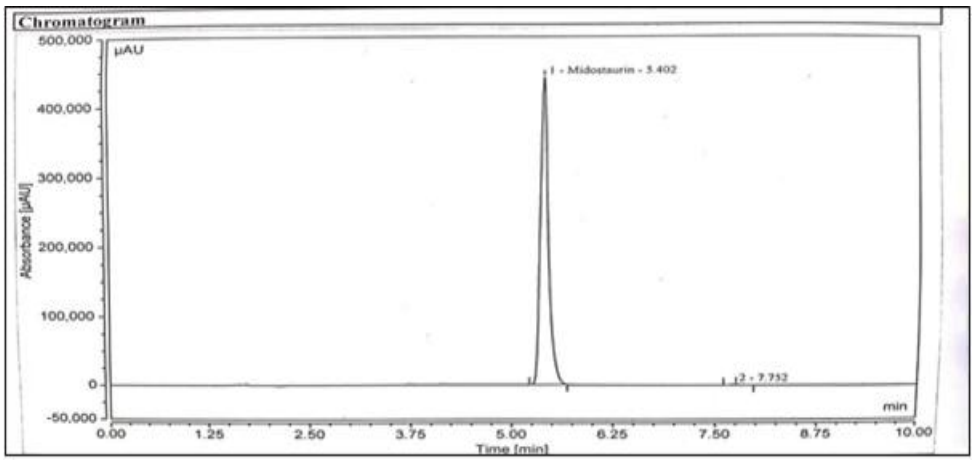

Fig 6: Chromatogram of Midostaurin Sample solution.

Linearity

Linearity was studied by preparing standard solution at six different concentration levels. The linearity range was found to be 10-80 ug/ml prepared from the standard stock solution of 500 ug/ml. A calibration curve was plotted by taking concentration level of drug on X-axis and the corresponding peak area on Y-axis and linearity was found to be 0.9994.The developed methodis showing good linearity over the range of 10-80ug/ml.

Table no.7: Linearity.

Linearity level	Concentration (ug/ml)	Peak Area
1	10	12056.5
2	20	23867
3	30	36591.5
4	50	58962.5
5	60	71188
6	80	92427

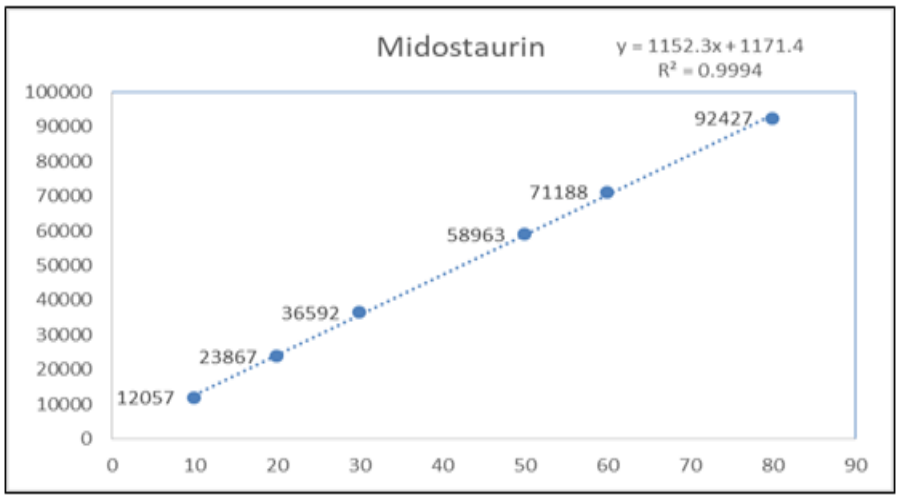

Fig 8: Linearity Curve of Midostaurin.

Precision

System Precision- six injection of standard solution (50ug/ml) were injected into the HPLC. The standard deviation and relative standard deviation of 6 replicate injections was calculated and reported.

Method Precision-6 injections of the sample solution (50ug/ml) were injected into the HPLC. Thestandard deviation and relative standard deviation of 6 replicate injections was calculated and reported.

Table no. 9(a): System precision of standard.

System Precision (Standard)	
Injection No.	Area at 293 nm
1	54282
2	54545
3	54405
4	54255
5	54255
6	54268
MEAN	54335
SD	117.5
% RSD	0.216
Limit	NMT 2.0%

Table no. 9 (b): Method precision of sample.

Method Precision (sample)	
Injection No.	Area at 293 nm
1	50999
2	50954
3	50885
4	50825
5	50971
6	50738
MEAN	50895
SD	99.72
%RSD	0.195
Limit	NMT 2.0%

Accuracy

Accuracy is defined as closeness of agreement between a measured quantity value and a true quantity value. Accuracy was determined by the method of recovery experiments, by the determination of % mean recovery of sample at 3 different levels (100,110 and 120%). At each level, three determination were performed. The Percentage recovery and standard deviation ofthe percentage recovery were calculated.

Table no. 10: Accuracy of standard solution.

ACCURACY				
% Level added	**STD Spiked (ug/ml)**	**Amount Recovered (mg)**	**% Recovery**	**Mean % Recovery**
110	5	27.43	99.74	99.64
110	5	27.40	99.65	
110	5	27.37	99.54	
120	10	30.45	101.52	101.77
120	10	30.55	101.83	
120	10	30.59	101.96	
130	15	31.88	98.11	98.10
130	15	31.86	98.03	
130	15	31.90	98.16	
			MEAN	99.84
			SD	1.8430
	Limit	NMT 2.0%	% RSD	1.8459

Robustness

The robustness of the method was established by deliberate change in detection wavelength by ±2nm, change in the Temperature by ±2c, change in mobile phase composition by ±5ml and flowrate by ±0.2ml in the estimation of capsule. The reproducible results were obtained which provesthat method is robust.

Table no.11: Robustness of Midostaurin.

ROBUSTNESS					
Parameter	**Change in parameter**	**% Estimation**	**Mean**	**SD**	**% RSD**
Wavelength (±2nm)	291	101.4	100.96	0.7505	0.7433
	293	100.1			
	295	101.4			
Temperature (±2°C)	28	101.4	100.88	0.8040	0.7970
	30	99.96			
	32	101.3			
Flowrate (±0.5 ml/min)	0.8	101.5	101.03	0.8082	0.8000
	1	100.1			
	1.2	101.5			
Mobile phase	25: 75	101.98	101.90	0.1588	0.15585
	20: 80	101.72			
	15: 85	102.00			
		limit			NMT 2.0%

Assay

Six sample preparation of Midostaurin were prepared and injected into the HPLC. The mean, standard deviation and % RSD of assay percentage of Midostaurin sample solution was calculated. The limit for assay is not less than 98% and not more than 102% of the labeled content.

Table no.7: Assay of Midostaurin.

ASSAY					
SR No.	**Weight of standard (mg)**	**Sample Weight (equivalent to 25 mg)**	**Area of standard at 293 nm**	**Area of sample at 293nm**	**% Assay**
1	10.8	1 capsule	54146	50999	101.7
2		1 capsule		50163	100.1
3		1 capsule		50116	99.96
4		1 capsule		50199	100.1
5		1 capsule		50738	101.2
6		1 capsule		50825	101.4
				Mean	100.74
				SD	0.7741
	Limit- 2.0%			%RSD	0.7684

RESULTS AND DISCUSSION

Novel and simple RP HPLC method have been developed for the determination of Midostaurin in Capsule dosage forms. The optimized chromatographic condition was found satisfactory to yield well retained, sharp and symmetrical peak at 10 min. The results of

linearity studied over the concentration range 10-80 µg/mL showed the linear detector response with correlationcoefficient of 0.9994.

Good recovery of the spiked drug was obtained by standard drug addition method at each added concentration, indicating that the method was accurate.

The method was sufficiently robust for normally expected variations in chromatographic conditions such as wavelength, temperature, flow rate and mobile phase.

For specificity study reveals that the peak obtained in the standard and sample chromatogram at working concentration are only because of the drug. In blank and excipient solution, there is no peak at the retention time of Midostaurin which proved that there was no interference of the blank and excipient peaks.

The number of Average theoretical plates was 15627 and tailing factor was 1.35 for Midostaurin,which indicates efficient performance of the column.

CONCLUSION

The developed HPLC method is simple, specific, accurate and precise for Midostaurin in capsule dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity and robustness in accordance with ICH Q2 (R1) guidelines. Also the developed method is better than earlier published articles with respect to superior System Suitability Parameter such as Theoretical plates, tailing factor. Thus the described method is suitable for routine analysis and quality control of Midostaurin in capsule dosage forms.

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