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Quantitative Estimation, Validation and Stability Indicating Assay Method for Determination of Related Substances in Midostaurin

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ABSTRACT

This study will help to develop the method, which is accurate, precise and cheaper than other developed methods, this method can be applied on industrial basis which will help in cost cutting of the drug manufacturing. This study also includes the estimation of impurity profile the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations

Keywords- Midostaurin, Anticancer, UV spectroscopy, HPLC, Validation, Stability studies.

INTRODUCTION

Anticancer Drugs were estimated by several analytical methods either alone or in combination by UV, Reverse HPLC, HPTLC, HPLC, LC-MS, MS, LC, Radioimmunoassay. In the view of the need in the industry for routine analysis anticancer drugs attempts are being made to develop simple and accurate instrumental methods for quantitative estimation of their determination in formulation. Thus there is a need for the development of newer, simple, sensitive, accurate and economic analytical methods for effective estimation of Anticancer Drugs. Analytical monitoring of a pharmaceutical product or specific ingredient in the product is necessary to ensure its safety, efficacy throughout all phases of its shelf life. [1]

MATERIALS AND METHOD

Selection of solvents

Methanol (AR grade) was selected as the solvent after considering the solubility and stability factor of both the drugs as well as the interference due to the excipients matrix present in the tablet formulation.

Preparation of stock solutions

To prepare stock solution of Midostaurin, (100 µg/ml) 100mg of Midostaurin was placed in 100 ml volumetric flask and dissolved in 75 ml of methanol and the volume was made up to the mark with methanol, to obtain the solution of 1000 µg/ml . 10 ml of the solution was diluted up to 100ml with methanol to produce final stock solution of 100 µg/ml of Midostaurin. [2]

Preparation of Standard for the test of linearity

From the stock solution of 100µg/ml appropriate dilution with methanol was made to prepare the solution with concentration. The absorbance was measured and the calibration curves were plotted from the mean values of observation.

Intermediate Precision (Inter-day and Intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution ones the same day and on different days at different time intervals respectively.

Limit of Detection and Limit of Quantitation (LOD) and (LOQ)

100 mg each of the reference standards of MID, were weight and transferred to separate 100 ml volumetric flasks. All three drugs were dissolved in methanol and the volume were made up to the mark with, the same solvent to get solutions of concentration 1000 µg/ml. 1 ml of each of these was diluted to 10 ml with

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methanol in two separate volumetric flasks to get solutions of concentration 100 µg/ml. For each drug appropriate aliquots were pipetted from the final solutions into a series of 10 ml volumetric flasks. The volumes were made with methanol to get a set up solutions of each drug in various concentration ranges (2, 4, 6 … 20 µg/ml for MID). The absorbance was measured at 249 nm. This was repeated ten times and the standard deviation of the analyte was calculated.[3]

Stability studies of midostaurin

Design

A minimum of four samples should be generated for every stress condition,

1. The blank solution is stored under normal conditions.
2. The blank subjected to stress in the same manner as the drug solution.
3. Zero-time sample containing the drug which is stored under normal conditions.
4. Drug solution subjected to stress treatment.

Chromatographic conditions used for the developed and validated HPTLC method for Midostaurin

The following densitometric conditions were used for HPTLC studies:

Stationary phase	: Pre-coated plates of Silica Gel 60 GF254 (Merck)
Mobile phase	: Chloroform: methanol: formic acid (8.2:1.5:1)
Saturation time	: 15 min
Development time	: 15 min
Wavelength	: 254 nm
Lamp	: Deuterium
Band width	: 7 mm
Length of chromatogram	: 8 cm

Forced Degradation Studies of Midostaurin[4]

Acid degradation

1 M of hydrochloric acid (HCl) was prepared by diluting 8.5 ml of concentrated HCl to 100 ml of distilled water. 1mg/ml solutions were prepared of Midostaurin. 1 ml of Midostaurin solution and 4 ml of 1N HCl were mixed and the mixture was refluxed ina water bath for 3 hours at 60°C. The refluxed solution of Midostaurin and HCl was allowed to attend ambient temperature and then the refluxed solution was neutralized by 1 N NaOH to pH 7 and the volume was made up to 10 ml with methanol. Then the final solution was applied to the TLC plates.

Total degradation was found when the Midostaurin was refluxed 1 N HCl for 3 hr., therefore the exposure time was reduced to 1 hr. with the same concentration of HCl. Then the stressed sample was analyzed. The chromatogram of the 1 hr. refluxed sample showed the same pattern of degradation as that of the 3-hr. refluxed sample. There wasa total of six peaks including the peak of Midostaurin at Rf 0.19 and other peaks were detected at Rf 0.11, 0.17, 0.34, 0.36, 0.49 (Fig.11.1).

Thus, the exposure time of the Midostaurinto HCl was kept for 1 hr.and the concentration of HCl was decreased to 0.1N. Further on analysis the stressed sample showed almost no change compared to the previous conditions. Hence it was concluded that the Midostaurin was not stable under any stressed acidic conditions tested.[5]

Base degradation

1M of NaOH was prepared by dissolving 4 g of sodium hydroxide pellets in 100 ml of distilled water.1 ml of Midostaurin solution (1 mg/ml) and 4 ml of 1N NaOH were mixed and the mixture was refluxed on water bath for 3 hours at 60°C. The solution was allowed to attend ambient temperature then the solution was neutralized by 1 N HCl to pH 7 and the volume was made up to 10 ml with methanol. Then the final solution was applied on to the TLC plates.Total degradation was found when the Midostaurin was refluxed 1 N NaOH for 3 hr,therefore the exposure time was reduced to 1 hr with the same concentration of NaOH. Then

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the stressed sample was analyzed. The chromatogram of the 1 hr refluxed sample showed the same pattern of degradation as that of the 3 hr refluxed sample. There wasa total of six peaks including the peak of Midostaurin at Rf 0.22 were detected and other peaks at Rf 0.09, 0.14, 0.23, 0.35, 0.54. Thus, the exposure time of the Midostaurin to NaOH was kept for 1 hr. and the concentration of NaOH was decreased to 0.1N. Further on analysis the stressed sample showed almost no change compared to the previous conditions. Hence it was concluded that the Midostaurin was not stable under any stressed alkaline conditions tested.[6]

Oxidative degradation

Oxidative degradation of Midostaurin was studied using 3% of H2O2 for 3 hr. 1 ml of Midostaurin solution (1 mg/ml) and 9 ml 3 % H2O2 solution were mixed and the mixture was refluxed ina water bath for 3 hr at 60°C.The solution was allowed to attend to ambient temperature and applied to the TLC plates. When the sample was analyzed, three additional peaks were obtained of degradants. There were no additional peaks at the same Rf when the untreated sample of Midostaurin was analyzed confirming the formation of three degradation products. Comparison of the peak area of Midostaurin in stressed condition with that of the untreated sample revealed a 35.66% decrease. The UV spectra confirmed the peak of Midostaurin in the stressed sample.[7]

Wet degradation

10 ml of aqueous Midostaurin solution (1 mg/ml) were refluxed on the water bath for 3 hr at 60°C. The solution was allowed to attend to ambient temperature and applied on to the TLC plates.There were four peaks of degradants beside the Midostaurin peak with the Rf values0.06, 0.36, 0.61, and 0.80. Midostaurin peak at Rf 0.21 was confirmed by comparing the UV spectrum with the untreated standard Midostaurin. The λmax of the tested Midostaurin (365) and even of the standard Midostaurin λmax was 365 nm. Thus it was confirmed that only 22.76% of Midostaurin was degraded. [8]

Dry heat

5mg of Midostaurin was placed in an oven for 3 hr at 100°C and then the heated sample of Midostaurin was dissolved in 5ml of methanol. 5mg of Midostaurin was placed in an oven for 24 hr at 60°C and then the heated sample was dissolved in 5ml of methanol. The solution was allowed to attend to ambient temperature and applied to the TLC plates.

When the stressed sample was analyzed, no degradation was found at both conditions. Hence the exposure time was increased up to 48 hr. and the temperature was kept constant at 60°C. When the stressed sample was analyzed, there were no additional peaks. The comparison between the peak areas of a stressed sample of Midostaurin with that of the untreated sample showed no difference. Hence it was concluded that the drug was stable under the tested conditions.[9]

Photo stability study

5mg of Midostaurin was exposed to UV short (254 nm) light for 24 hr. Then the exposed sample of Midostaurin was dissolved in 5ml of methanol and applied to the TLC plates.The stress study under short UV showed two additional peaks with Rf 0.12,0.64 (Fig.11.8). There were no additional peaks at the same Rf of standard when the untreated sample was analyzed confirming the formation of two degradation products. Comparison of the peak area of Midostaurin in stressed conditions with that of the untreated sample revealed an 18.47 % decrease in the peak area of Midostaurin under short UV. The UV spectra of the peak with Rf 0.12 in the stressed sample matched with the untreated Midostaurin and confirmed the presence of Midostaurin. Thus, it was concluded that 18.47% of Midostaurin was degraded upon UV exposure.[10]

Table 1 Linearity studies of Midostaurin at 249 nm

S. No.	Conc.	Absorbance of Midostaurin	SD(±)

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	(µg/ml)	Replica 1	Replica 2	Replica 3	Replica 4	Replica 5	Mean	
1	2	0.184	0.178	0.180	0.173	0.181	0.179	0.00409
2	4	0.374	0.355	0.360	0.364	0.356	0.362	0.00769
3	6	0.588	0.575	0.572	0.586	0.578	0.579	0.00694
4	8	0.713	0.734	0.713	0.719	0.728	0.723	0.00934
5	10	0.892	0.906	0.904	0.916	0.912	0.906	0.00917
6	12	1.063	1.066	1.064	1.068	1.078	1.068	0.00602
7	14	1.257	1.267	1.269	1.269	1.284	1.269	0.00965
8	16	1.448	1.41	1.437	1.452	1.432	1.442	0.01653
9	18	1.601	1.604	1.596	1.584	1.605	1.597	0.00857
10	20	1.804	1.814	1.796	1.808	1.798	1.807	0.00735

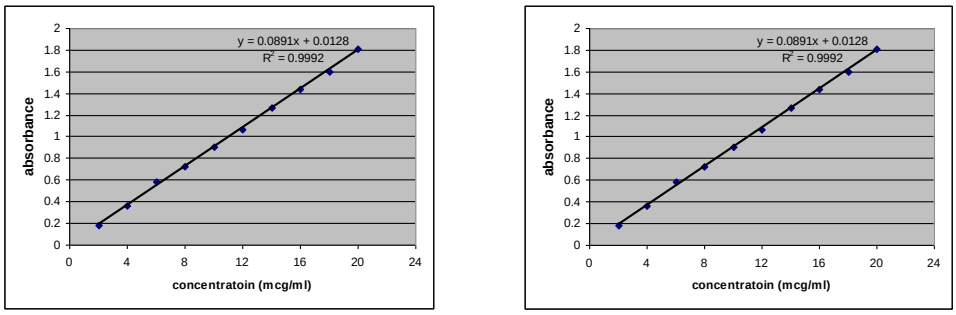

Fig 1 a) Linearity studies of Midostaurin at 249 nm b)LOD and LOQ of Midostaurin

Table 2: Intra and inter day precision study of Midostaurin.

Replicate No.	Intraday			Interday		
	Percentage obtained			Percentage obtained		
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate-1	99.12	99.87	99.12	100.12	98.10	99.86
Replicate-2	99.53	99.90	98.90	99.97	99.13	98.97
Replicate-3	99.67	99.45	98.97	100.13	99.01	99.98
Replicate-4	99.01	99.01	98.12	99.34	98.12	98.98
Replicate-5	98.87	99.39	98.98	100.06	99.12	99.67
Mean	99.24	99.52	99.81	99.92	98.69	99.49
S.D.	0.344	0.370	0.398	0.333	0.537	0.485
%CV	0.35	0.37	0.40	0.33	0.54	0.49

Table 3: LOD and LOQ of Midostaurin

S. No.	Conc. (µg/ml)	Absorbance of Midostaurin							SD(±)
		Replica 1	Replica 2	Replica 3	Replica 4	Replica 5	Replica 6	Mean	
1	2	0.184	0.178	0.180	0.173	0.181	0.182	0.179	0.00409
2	4	0.374	0.355	0.360	0.364	0.356	0.370	0.362	0.00769
3	6	0.588	0.575	0.572	0.586	0.578	0.584	0.579	0.00694
4	8	0.713	0.734	0.713	0.719	0.728	0.713	0.723	0.00934
5	10	0.892	0.906	0.904	0.916	0.912	0.904	0.906	0.00917
6	12	1.063	1.066	1.064	1.068	1.078	1.064	1.068	0.00602
7	14	1.257	1.267	1.269	1.269	1.284	1.267	1.269	0.00965

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8	16	1.448	1.41	1.437	1.452	1.432	1.41	1.442	0.01653
9	18	1.601	1.604	1.596	1.584	1.605	1.604	1.597	0.00857
10	20	1.804	1.814	1.796	1.808	1.798	1.814	1.807	0.00735

Mean standard deviation	0.0085
Slope	0.0891
LOD	3.3 x 0.0085/0.0891= 0.32µg/ml
LOQ	10 x 0.0085/0.0891= 0.95 µg/ml

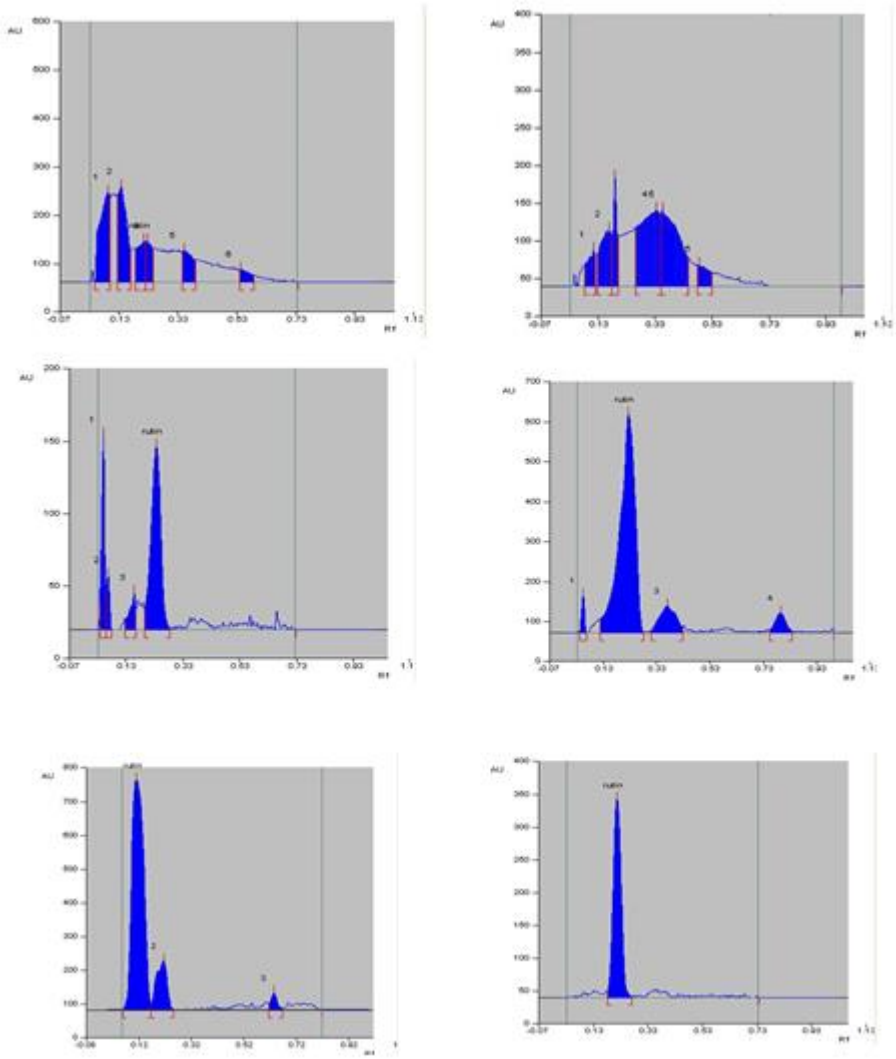

Fig. 2: HPTLC Chromatogram of Midostaurin a) after acid degradation b) after base degradation c)after oxidative stress d) after wet degradation e) after dry degradation f) after UV exposure

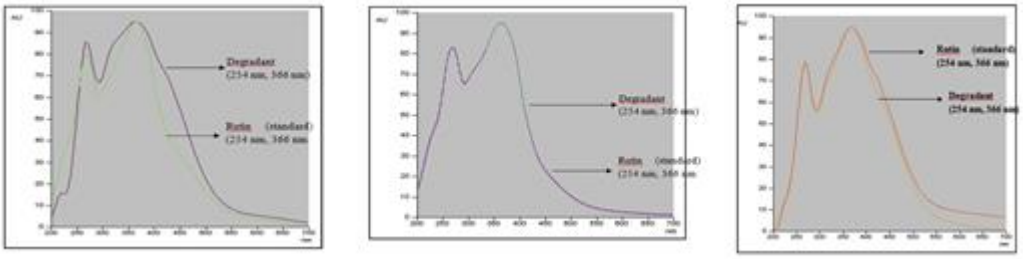

Fig. 3: UV spectrum of Midostaurin (standard) and degraded product a) after oxidative stress b) after wet degradation c) after UV exposure

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CONCLUSION

The application of developed and validated HPTLC methods for Midostaurin as stability indicating methods was successfully employed. It was observed that Midostaurin was stable only under dry conditions but the other conditions had altered the concentration of Midostaurin. Midostaurin is a biomarker with very good anti-oxidants and many other therapeutic activities. It is even present in many medicinal plants. Thus, the stability indicating method can be very well adapted for the evaluations of many different formulations containing these two biomarkers.

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