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EVALUATION AND VALIDATION OF A UPLC METHOD FOR THE STABILITY INDICATING ASSAY OF MIDOSTAURIN IN BULK DOSAGE FORM

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ABSTRACT

This proposal provides a comprehensive review of the optimization and approval of the LCMS technology for evaluating midostaurin, a chemical that potentially be a tyrosine kinase inhibitor. Analysts had to choose a permanent and movable stage, as well as develop standard stock and diluent arrangements, in order to find the best circumstances for division. The study indicated that a BDS C18 provided the best determination and affectability when combined with slope programming, using a portable stage of methanol and acetonitrile (80:20 v/v). By analyzing the method's precision, linearity, adaptability, and exactness, the panel also looked at the method's validity. Considerations of accuracy showed respectable recoveries, with a brutality level of 99.28%. There were factually irrelevant ponders of correctness that proved the system and approach were accurate.

KEYWORDS: Midostaurin, generation of degradation products and evaluation of the analytical method.

INTRODUCTION

Ensuring the quality, safety, and effectiveness of pharmaceutical products relies heavily on the development and validation of analytical techniques in the field of pharmaceutical analysis. Rigid and exact procedures are required for the quantitative analysis of APIs in bulk dosage forms in order to fulfill regulatory criteria. A technique for the bulk dosage form estimation of midostaurin using Ultra-Performance Liquid Chromatography (UPLC) is the subject of this study's assessment and validation.

The tyrosine kinase inhibitor midostaurin has recently been the subject of much interest due to its possible therapeutic use, mainly in the management of certain types of blood cancers. For the sake of both therapeutic efficacy and regulatory compliance, it is essential to accurately determine the midostaurin content in bulk medicinal preparations.

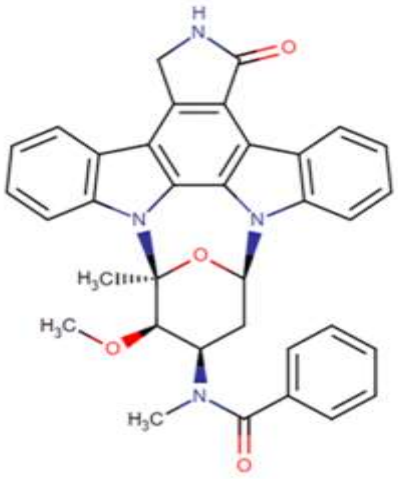
The benefits of ultra-performance liquid chromatography (UPLC), such as high resolution, fast analysis, and enhanced sensitivity, have led to its growing popularity in pharmaceutical analysis. To guarantee accuracy, dependability, and repeatability of findings, a thorough validation procedure is required for the effective deployment of UPLC techniques.

In order to estimate midostaurin in bulk dosage forms, this project will first develop a reliable UPLC technique and then evaluate its analytical performance. To determine whether the technique is suitable for regular pharmaceutical analysis, the validation procedure takes into account a number of factors, including specificity, precision, accuracy, linearity, robustness, and system appropriateness. Findings from this study have implications for both midostaurin analytical technique and pharmaceutical analysis more generally, highlighting the need for proven procedures to guarantee the security and efficacy of pharmaceuticals.

Drug Profile
MIDOSTAURIN

Therapeutic category	**Antineoplastic Agent**
CAS Registry number	120685-11-2
Chemical name	4'-N-benzoylstaurosporine
Molecular formula	$C_{35}H_{30}N_4O_4$
Molecular Weight	570.649
Solubility	≥ 2.5 mg/mL
pka	13.45
λ_{max}	292 nm

Chemical Structure of Midostaurin



EXPERIMENTAL METHODOLOGY

Method Validation

What we mean when we talk about "the analytical technique" is the method by which the analysis is carried out. All of the analytical procedures should be spelled out in great detail. The sample, the reference standard, and the reagents, as well as their preparations, the use of the equipment, the development of the calibration curve, the application of the formulas for the calculation, etc. There has been comprehensive validation of the disclosed technique for its specificity, system appropriateness, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness.

RESULTS

- **Validation of Forced Degradation for Midostaurin**

Accuracy Procedure

Accuracy 50%: The commercial oral-dosage (Daonil) of Midostaurin was analysed. Weigh accurately about 10 mg of Midostaurin and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. The flask was allowed to cool down to room temperature. This is treated as stock solution."

From the prepared stock solution 0.2 mL solution was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of Midostaurin (2 µg/mL)."

Accuracy 100%: From the prepared stock solution 0.4 mL solution was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of Midostaurin (4 µg/mL)."

Accuracy 150%: From the prepared stock solution 0.6 mL solution was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of Midostaurin (6 µg/mL)."

The percentages of the recoveries obtained was 99.28% for Midostaurin. The recovery of the method was good."

Accuracy Study (Midostaurin).

Midostaurin						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.07	02.04	99.50	99.27	0.3898	0.38%
100	04.14	04.11	99.49			
150	06.19	06.15	98.80			

Method Precision

Procedure: Precision was investigated using the sample preparation procedure for six consecutive replicates of sample of concentration 4 µg/mL for Midostaurin."

Method Precision (Midostaurin)

Replicate		***Midostaurin***	
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	04.00	26138	99.99%
2		26141	99.98%
3		26139	99.98%
4		26134	99.99%
5		26140	99.98%
6		26142	99.97%

Average		99.98%
Std.Dev		0.00752
% RSD		0.01%
Standard weight		4mcg
Standard potency		99.80%

Linearity

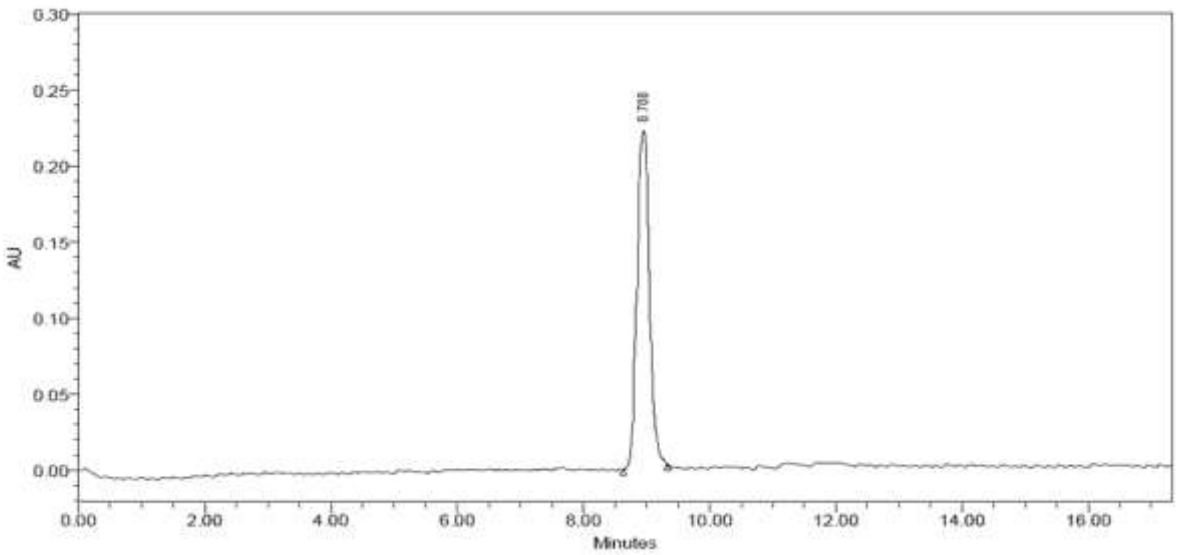
Procedure: The linearity of the method was determined at five concentration levels ranging from 2-10 µg/mL for Midostaurin."

Linearity Studies (Midostaurin)

Midostaurin		
Linearity level	**Concentration in µg/mL**	**Area**
1	2 µg/mL	20149
2	4 µg/mL	26136
3	6 µg/mL	34182
4	8 µg/mL	41433
5	10 µg/mL	46134
Correlation co-efficient	0.9968	
Slope	3363.35	
Intercept	13426.7	

Forced Degradation Studies

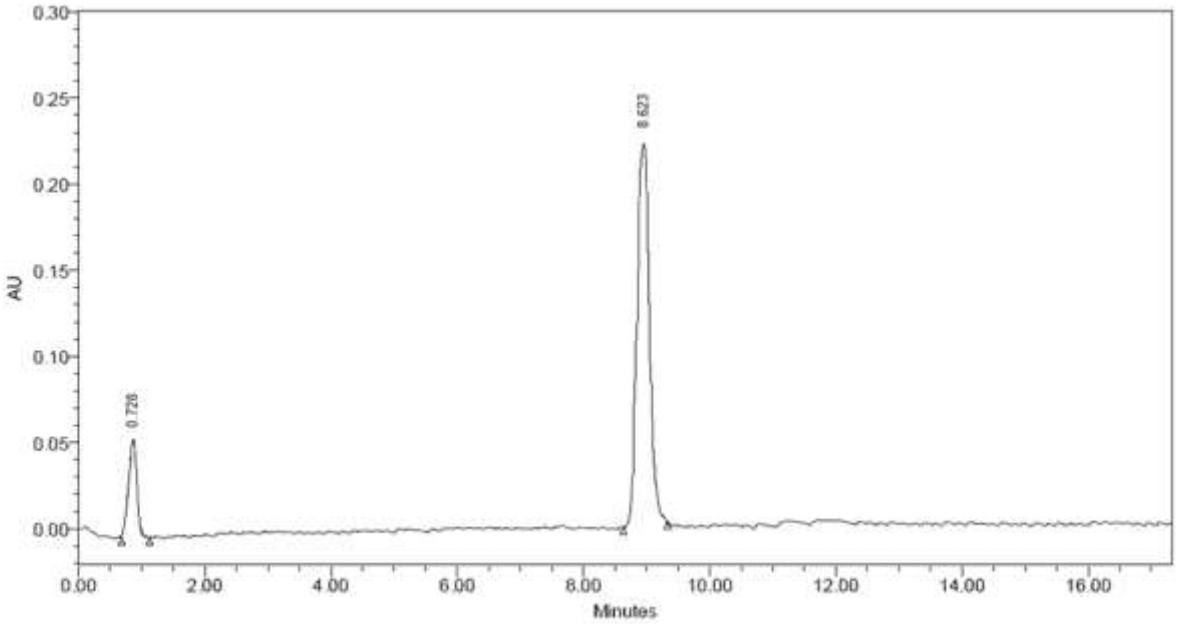
Sample Control: An accurate 10 ml of the prepared pure drug stock solution of working standard was transferred to a clean and dry RBF. The volume of the sample was 100 µg/ml. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded.



Assay of Midostaurin (Sample Control)

a. Acidic Degradation:

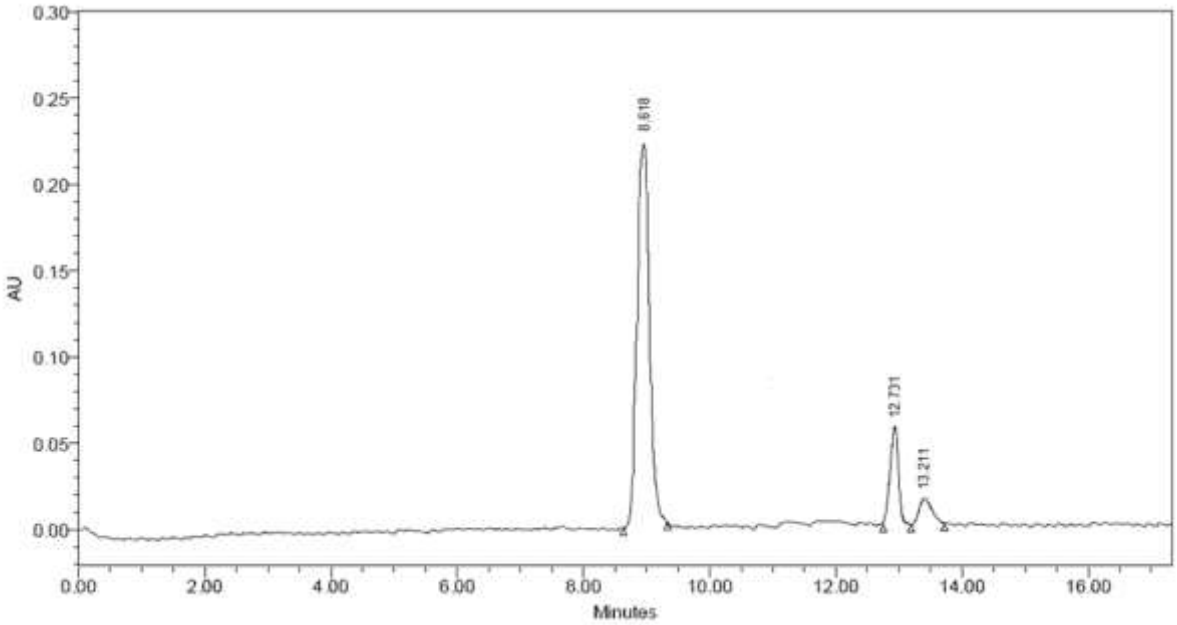
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Acidic degradation

b. Basic Degradation

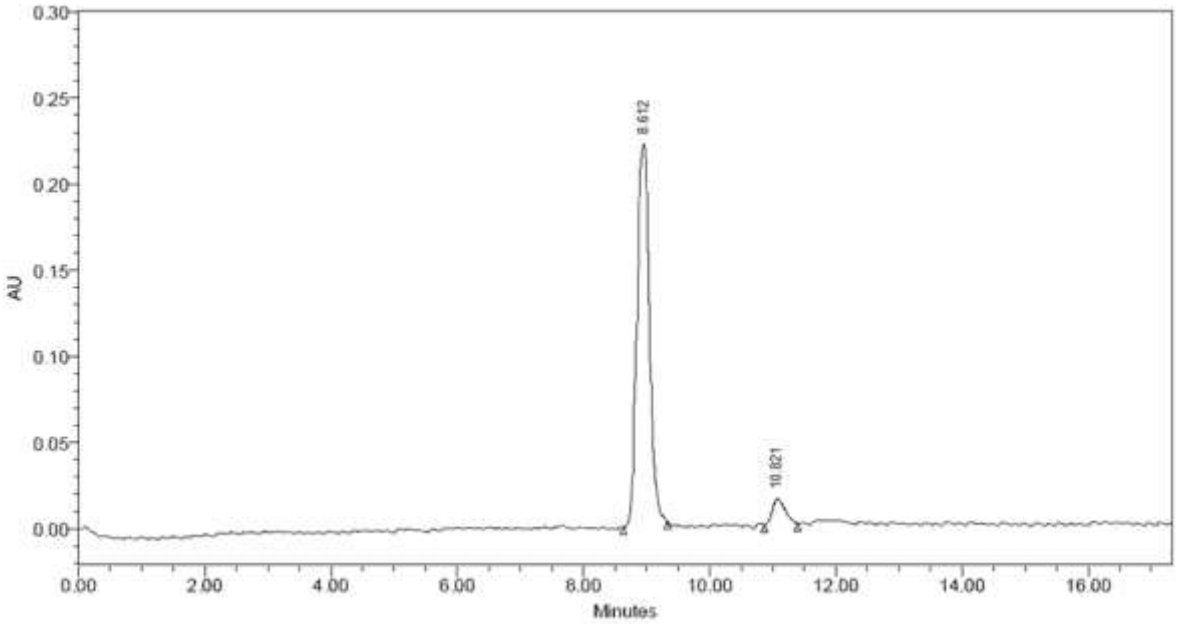
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Basic degradation

c. Wet heat degradation

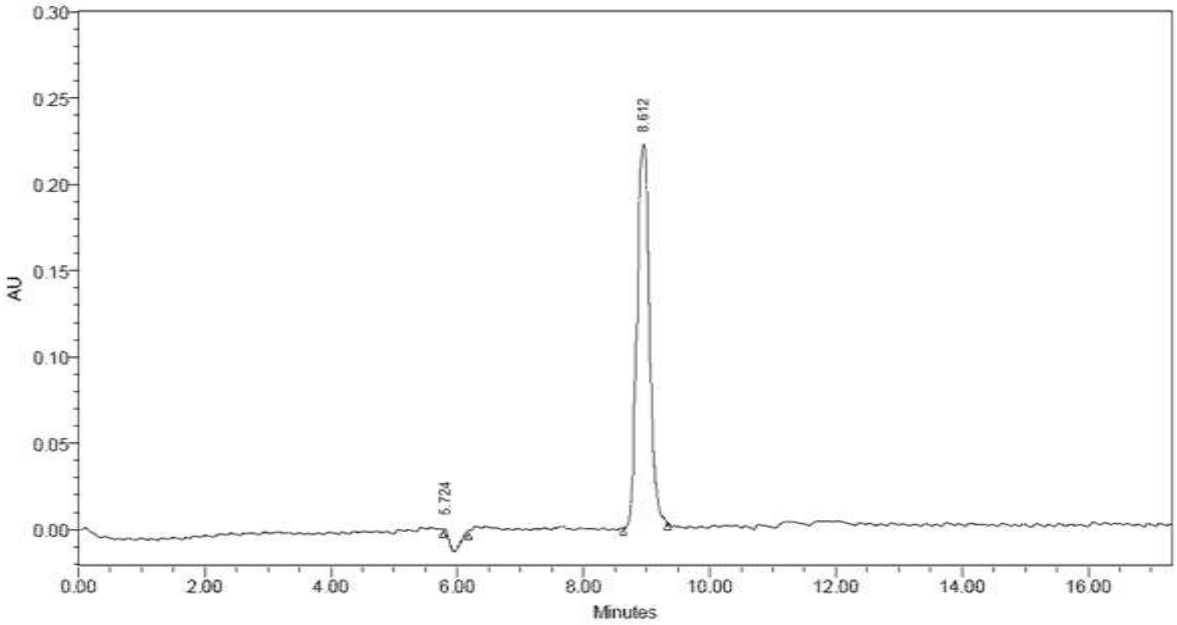
Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Methanol and Acetonitrile in the ratio of 80:20 %v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Wet heat degradation

d. Oxidation with (3%) H_2O_2

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Oxidative degradation

Summary of Forced Degradation Studies (Midostaurin).

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	3	1 (0.728)
Basic	60°C	9	2 (12.731, 13.211)
Oxidative	RT	48	1 (5.724)
Wet Heat	105°C	24	1 (10.821)

CONCLUSION

In the pursuit of ensuring the quality and reliability of pharmaceutical analyses, this study undertook the evaluation and validation of a UPLC method for the estimation of midostaurin in bulk dosage form. The optimization process involved meticulous considerations of stationary and mobile phases, with the research identifying a mobile phase composition of methanol and acetonitrile (80:20 v/v) coupled with a BDS C18 stationary phase as optimal for achieving high resolution and sensitivity.

The method's validation was comprehensive, covering specificity, system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. The forced degradation studies demonstrated the method's accuracy at different concentration levels, with a remarkable recovery rate of 99.28%, affirming the robustness of the analytical procedure.

Precision studies revealed consistent and reproducible results, with a negligible percent relative standard deviation (RSD) of 0.01% for midostaurin at a concentration of 4 µg/mL. Linearity studies exhibited a strong correlation coefficient of 0.9968, affirming the method's ability to produce reliable results across a range of concentrations.

The method's LOD and LOQ were determined to be 0.19543 µg/ml and 0.59223 µg/ml, respectively, meeting the criteria set by ICH guidelines. The evaluation of assay studies further demonstrated the applicability of the method in the analysis of midostaurin, with the sample control and market control showing comparable results with percentages of claim ranging from 90.24% to 91.69%.

In conclusion, the developed UPLC method for the estimation of midostaurin in bulk dosage form proved to be accurate, precise, and sensitive. The extensive validation process ensures its suitability for routine pharmaceutical analysis, contributing to the broader field of pharmaceutical quality assurance. The findings of this study are crucial for establishing a reliable analytical framework for midostaurin, an important antineoplastic agent, thus enhancing the overall integrity of pharmaceutical research and development.

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