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EVALUATION AND VALIDATION OF A UPLC METHOD FOR THE ESTIMATION OF MIDOSTAURIN IN BULK DOSAGE FORM

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ABSTRACT

Midostaurin could be a tyrosine kinase inhibitor, and this proposal gives a total examination on the optimization and approval of the LCMS technique for analyzing this compound. To discover the most excellent conditions for division, analysts had to prepare standard stock and diluent arrangements and select a stationary stage and portable stage. Employing a portable stage of methanol and acetonitrile (80:20 v/v), the inquire about found that a BDS C18 given the ideal determination and affectability when coupled with slope programming. The ponder too examined the method's legitimacy by looking at its exactness, exactness, linearity, and flexibility. Thinks about of exactness appeared respectable recuperations, with a cruel of 99.28%. Ponders of accuracy demonstrated the system's and method's accuracy that were factually immaterial.

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

INTRODUCTION

In the realm of pharmaceutical analysis, the development and validation of analytical methods play a pivotal role in ensuring the quality, safety, and efficacy of pharmaceutical products. The quantification of active pharmaceutical ingredients (APIs) in bulk dosage forms demands rigorous and precise methodologies to meet regulatory standards. This study focuses on the evaluation and validation of a method utilizing Ultra-Performance Liquid Chromatography (UPLC) for the estimation of midostaurin in its bulk dosage form.

Midostaurin, a tyrosine kinase inhibitor, has garnered significant attention in recent years for its therapeutic potential, particularly in the treatment of certain hematological malignancies. Accurate determination of the concentration of midostaurin in bulk drug substances is imperative for maintaining the desired therapeutic effect while ensuring compliance with regulatory requirements.

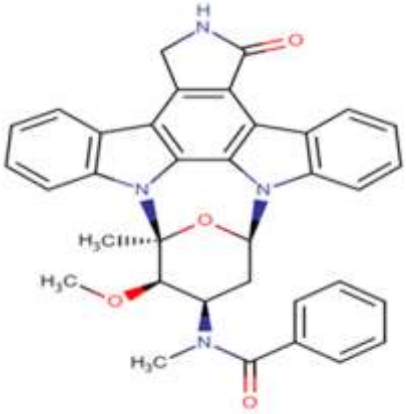
The use of UPLC in pharmaceutical analysis has become increasingly popular due to its advantages, including high resolution, rapid analysis, and improved sensitivity. However, the successful application of UPLC methods necessitates a comprehensive validation process to ensure reliability, accuracy, and reproducibility of results.

This study aims to present a robust UPLC method for the estimation of midostaurin in bulk dosage forms and subsequently validate its analytical performance. The validation process encompasses various parameters, such as specificity, precision, accuracy, linearity, robustness, and system suitability, to ascertain the method's suitability for routine pharmaceutical analysis. The outcomes of this research contribute not only to the analytical methodology for midostaurin but also to the broader field of pharmaceutical analysis, emphasizing the importance of validated methods in ensuring the quality and safety of pharmaceutical products.

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	$\geq$ 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."

Midostaurin						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std. Dev**	**% RSD**
50	02.06	02.05	99.51	99.28	0.3897	0.39%
100	04.12	04.10	99.50			
150	06.18	06.17	98.83			

Accuracy Study (Midostaurin)

Method Precision

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

Method Precision (Midostaurin)

Linearity

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

Linearity Studies (Midostaurin)

LOD and LOQ

Procedure

The limit of detection and limit of quantification were evaluated by serial dilutions of Midostaurin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ as per ICH guidelines."

Calculations of LOD and LOQ

Slope = a; Intercept = b; The number of tests = N

Standard Error (SE) of Intercept = EXCEL function data analysis → Regression → Table

SD of Intercept = SE of Intercept / Square root of N

LOD

LOD=3.3(SD of intercept/Slope)

Total numbers: 5

SE of Intercept: 477.198

SD of Intercept: 199.19

LOD= 3.3*(199.19/ 3363.35)

LOD= 3.3*(0.059223)

LOD= 0.19543(µg/ml)

LOQ

LOQ=10*(SD/S)

LOQ= 10*(199.19/ 3363.35)

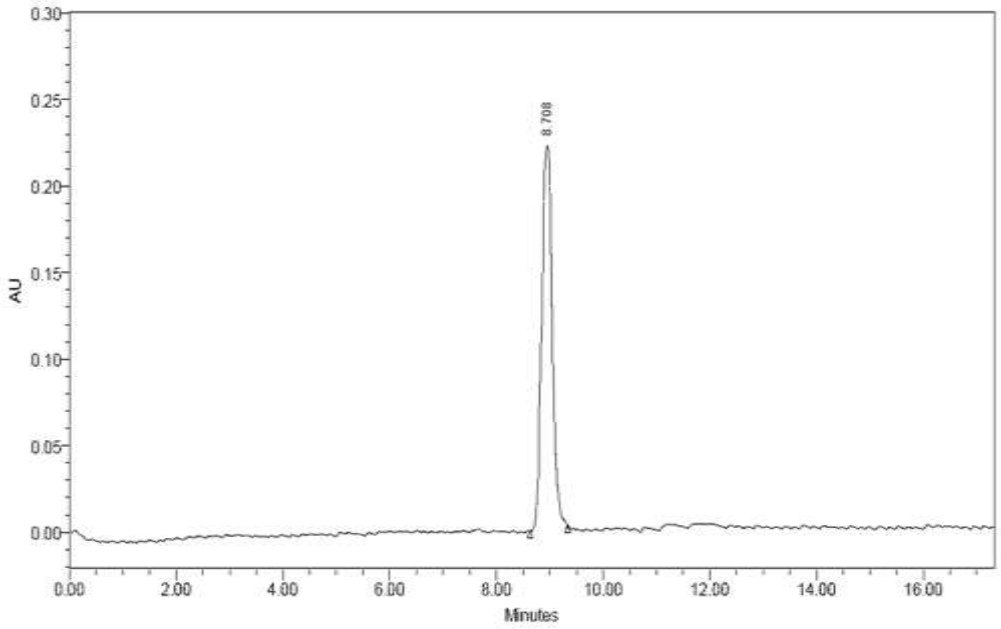
LOQ= 0.59223(µg/ml)

EVALUATION OF METHODS

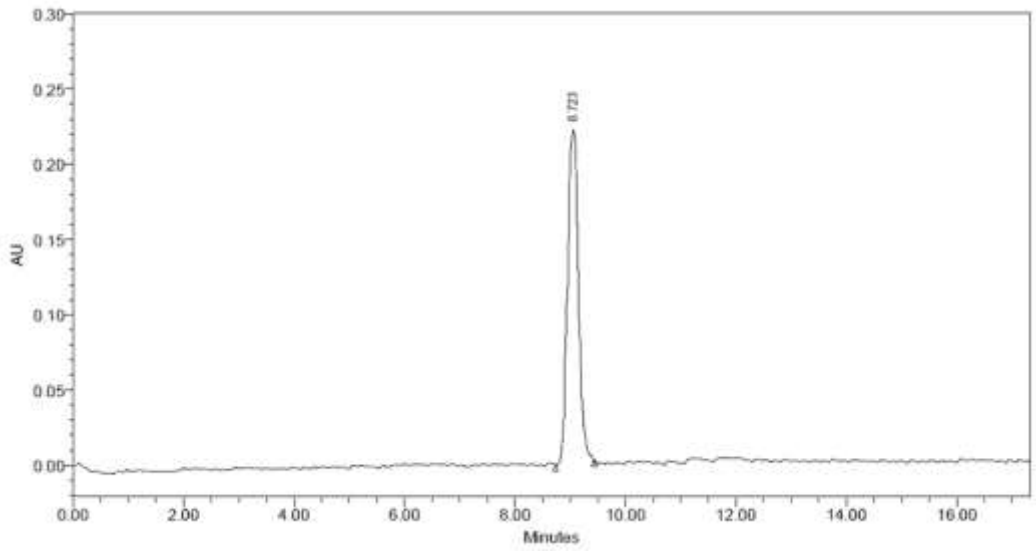
Assay Studies

Analysis of Midostaurin

Conditions	Sample Amount (µg/ml)	Peak Area	% claim
Sample Control	04.15	26139	91.69%
Market	04.12	26018	90.24%



Assay of Midostaurin (Sample Control)



Assay of Midostaurin (Market Control)

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 4µg/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 0.19543µg/ml and 0.59223µg/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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