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METHOD OPTIMIZATION AND VALIDATION OF MIDOSTAURIN USING LCMS

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Abstract -Midostaurin (PKC412) is an orally bioavailable multi-kinase inhibitor approved for the treatment of FLT3-mutated Acute Myeloid Leukemia (AML) and advanced systemic mastocytosis. Due to its high lipophilicity ($\log P \sim 4.6$) and narrow nanogram-level therapeutic window, establishing a sensitive and robust analytical method is essential. This study demonstrates the development, optimization, and validation of a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method for Midostaurin compliant with ICH Q2(R2) guidelines. Chromatographic separation was achieved on a Hypersil GOLD C18 column ($100\text{ mm} \times 2.1\text{ mm}$, $1.8\,\mu\text{m}$) using an isocratic mobile phase of 0.1% v/v formic acid in water and acetonitrile (25:75 v/v) at a flow rate of 0.30 mL/min. Mass spectrometric detection was performed in ESI+ mode utilizing Multiple Reaction Monitoring (MRM) targeting $m/z\ 571.20 \to 398.20$ for Midostaurin and $m/z\ 576.25 \to 310.10$ for Midostaurin-d_5 (Internal Standard). Midostaurin eluted at 2.15 min with a total run time of 3.50 min. High linearity was obtained over 0.50 ng/mL to 100.00 ng/mL ($r^2 = 0.9994$). Limits of Detection and Quantitation were 0.15 ng/mL and 0.50 ng/mL, respectively. Forced degradation testing per ICH Q1A(R2) demonstrated the stability-indicating nature of the method.

Key Words:Midostaurin, LC-MS/MS, Method Validation, ICH Q2(R2), Forced Degradation, Acute Myeloid Leukemia

1.INTRODUCTION

Acute Myeloid Leukemia (AML) is a malignant hematopoietic disorder where somatic mutations in the FMS-like tyrosine kinase 3 (FLT3) gene occur in approximately 30% to 35% of adult patients. Midostaurin is an N-benzoyl derivative of staurosporine approved by the US FDA and EMA in combination with chemotherapy for FLT3-mutated AML and as monotherapy for systemic mastocytosis.

Traditional HPLC-UV methods present limits of quantitation ($\text{LOQ} > 25.0\text{ ng/mL}$) inadequate for trace level evaluation and suffer from long analysis times. Coupling UHPLC with tandem mass spectrometry operating in Multiple Reaction Monitoring (MRM) mode yields superior selectivity, sub-nanogram sensitivity, and high-throughput capabilities

Table -1: Physico-Chemical Profile of Midostaurin

Parameter	Property / Specification
IUPAC Name	*N*-[(9*S*,10*R*,11*R*,13*R*)-2,3,10,11,12,13-hexahydro-10-methoxy-9-methyl-1-oxo-9,13-epoxy-1*H*,9*H*-diindolo[1,2-a:2',1'-p]pyrrolo[3,4-l][1,6,13]benzotriazacyclododecin-11-yl]-*N*-methylbenzamide PDF
Molecular Formula	$C_{35}H_{30}N_4O_4$ PDF
Molecular Weight	570.65 g/mol PDF
Partition Coefficient	$\log P = 4.60$ PDF
Aqueous Solubility	Practically insoluble (< 0.001 mg/mL at 25°C) PDF

2.1 Reagents and Instrumentation

Midostaurin (99.8% purity) and Midostaurin-d_5 standards were utilized alongside LC-MS grade acetonitrile, methanol, and formic acid. An Ultra-High Performance Liquid Chromatography system interfaced with a Triple Quadrupole Mass Spectrometer equipped with an Electrospray Ionization (ESI) source was employed.

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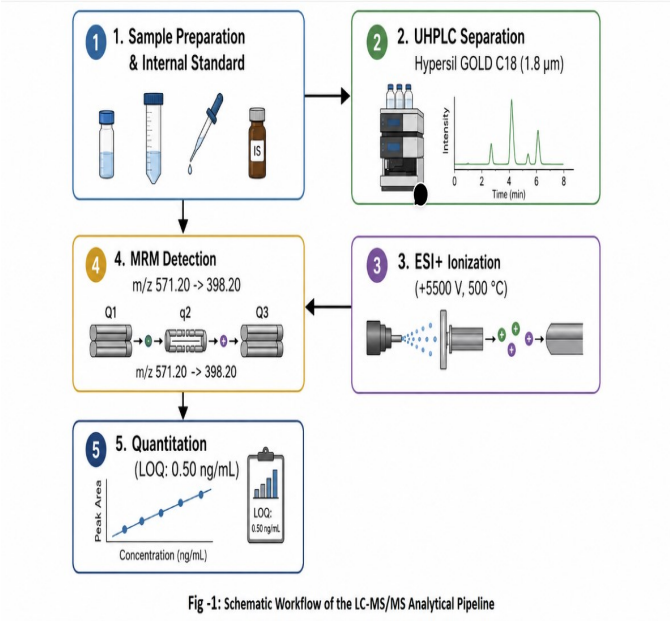
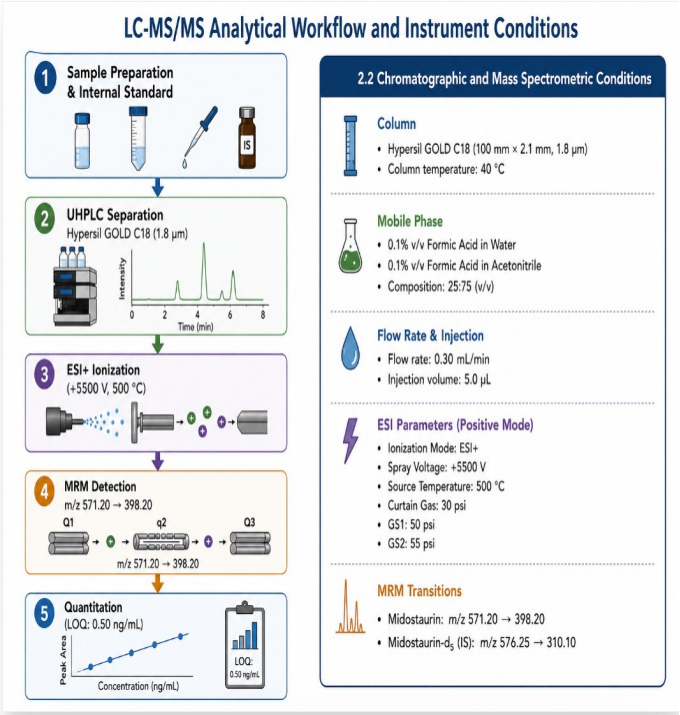


Fig -1: Schematic Workflow of the LC-MS/MS Analytical Pipeline



2.2 Chromatographic and Mass Spectrometric Conditions

Chromatographic separation and mass spectrometric detection were optimized to achieve rapid, selective, and sensitive quantification of Midostaurin. The optimized analytical conditions provided excellent peak shape, high signal intensity, and reproducible retention times, ensuring reliable performance throughout the method validation studies.

- **Column & Flow:** Hypersil GOLD C18 (100 mm × 2.1 mm, 1.8 µm), maintained at **40°C**; flow rate **0.30 mL/min**; injection volume **5.0 µL**.
- **Mobile Phase:** Isocratic mixture of **0.1% (v/v) Formic Acid in Water** and **0.1% (v/v) Formic Acid in Acetonitrile** in a **25:75 (v/v)** ratio.
- **Mass Spectrometric Parameters (ESI+):** Positive electrospray ionization (ESI+) with spray voltage **+5500 V**, source temperature **500°C**, curtain gas **30 psi**, GS1 **50 psi**, and GS2 **55 psi**.
- **MRM Transitions:** Midostaurin (**m/z 571.20 → 398.20**) and Midostaurin-d$_5$ (Internal Standard) (**m/z 576.25 → 310.10**) were monitored in Multiple Reaction Monitoring (MRM) mode for highly selective and sensitive quantification.

2.3 Method Validation and Forced Degradation

Method validation was performed according to ICH Q2(R2) guidelines to evaluate specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness. Forced degradation studies were carried out in accordance with ICH Q1A(R2) to confirm the stability-indicating capability of the developed LC-MS/MS method. Midostaurin exhibited the highest degradation under basic hydrolysis (24.80%), followed by oxidative stress (18.35%) and acidic hydrolysis (11.45%). The drug remained comparatively stable under photolytic (4.10%) and thermal stress (3.20%) conditions. Table 2. Accuracy and Precision Assessment across Quality Control Levels

Table 2. Accuracy and Precision Assessment across Quality Control Levels

QC Level	Nominal Conc. (ng/mL)	Intra-Day Mean ± SD (ng/mL)	Intra-Day % Recovery	Intra-Day % RSD	Inter-Day Mean ± SD (ng/mL)	Inter-Day % Recovery	Inter-Day % RSD
LQC	1.50	1.48 ± 0.04	98.67%	2.70%	1.47 ± 0.05	98.00%	3.15%
MQC	40.00	40.42 ± 0.65	101.05%	1.61%	40.18 ± 0.82	100.45%	2.04%
HQC	80.00	79.12 ± 1.18	98.90%	1.49%	79.35 ± 1.44	99.19%	1.82%

Table 3. Forced Degradation Stress Testing Results

Stress Condition	Parameters Applied	% Degradation	Degradant Rt (min)	Mass Balance (%)
Acid Hydrolysis	0.1 N HCl, 60°C, 2 h	11.45%	1.24	98.8%
Base Hydrolysis	0.1 N NaOH, 60°C, 2 h	24.80%	0.98, 1.45	97.6%
Oxidative Stress	3% H_2O_2, RT, 4 h	18.35%	1.15	99.1%
Thermal Stress	Dry Heat 80°C, 24 h	3.20%	None detected	99.4%
Photolytic Stress	200 Wh/m², 48 h	4.10%	1.32	98.9%

3. CONCLUSIONS

A rapid, ultra-sensitive, and stability-indicating LC-MS/MS method was successfully developed and validated for Midostaurin according to ICH Q2(R2) guidelines. The method demonstrated excellent selectivity, precision, accuracy, and robustness with a total chromatographic run time of 3.50 minutes and a lower limit of quantification (LOQ) of 0.50 ng/mL. These characteristics make the method highly suitable for routine quality control, stability studies, and bioanalytical applications.

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REFERENCES

1. International Council for Harmonisation (ICH). (2023). ICH Guideline Q2(R2): Validation of Analytical Procedures.
2. Bourget, P., et al. (2014). Liquid chromatography–tandem mass spectrometry assay for therapeutic drug monitoring of Midostaurin in plasma. Journal of Chromatography B, 944, 175–181.
3. Reddy Saddala, M. P., et al. (2022). Development and validation of a novel RP-HPLC method for Midostaurin determination using AQbD. Biomedical Chromatography, 36(12), e5486.
4. Attwa, M. W., et al. (2023). Development and validation of a rapid LC-MS/MS method for quantifying small-molecule kinase inhibitors. Molecules, 28(5), 2368.

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