RESEARCH ARTICLE

Stability-Indicating RP-HPLC Method Development and Validation for the Estimation of Mirdametinib in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A novel, stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Mirdametinib in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on an Agilent Eclipse XDB C18 column (150 × 4.6 mm, 3.5 µm) using an isocratic mobile phase comprising acetonitrile and 0.1% formic acid (30:70 v/v). Detection was performed at 223 nm with a flow rate of 1 mL/min and an injection volume of 10 µL. The method was validated in accordance with ICH Q2(R1) guidelines, confirming its specificity, precision, linearity, accuracy, robustness, and sensitivity. Linearity was demonstrated across the concentration range of 25–150 µg/mL with a correlation coefficient (R^2) of 0.99979. The system and method precision showed %RSD values below 2.0, while recovery studies confirmed accuracy with values between 99.8% and 100.5%. The limit of detection (LOD) and quantification (LOQ) were 0.60 µg/mL and 2.0 µg/mL, respectively. Robustness studies confirmed the method's reliability under slight variations in chromatographic conditions. Forced degradation studies under acidic, basic, oxidative, thermal, photolytic, and hydrolytic stress conditions demonstrated the method's capacity to differentiate Mirdametinib from its degradation products. The developed method was successfully applied to the assay of a marketed formulation (GOMEKLI), showing a 99.8% assay value. Overall, the validated RP-HPLC method offers a reliable and precise analytical tool for routine quality control of Mirdametinib.

Keywords: Mirdametinib, RP-HPLC, Method Validation, Forced Degradation, Stability-Indicating Method, Pharmaceutical Dosage Form

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INTRODUCTION

Mirdametinib (Fig.1), also known by its code PD-0325901, is a selective and orally active inhibitor of MEK1/2, primarily used for treating tumors linked to the RAS/RAF/MEK pathway, such as neurofibromatosis type 1 (NF1)-associated plexiform neurofibromas[1,2]. It functions by inhibiting the MEK enzymes, thereby disrupting the MAPK/ERK signaling cascade frequently overactivated in various cancers[3-5].

The compound has a molecular weight of 482.19 g/mol and a formula of $C_{16}H_{14}F_3IN_2O_4$. Its logP value of approximately 2.8 indicates a moderate lipid solubility, and its pKa is around 8.4. Although poorly soluble in water, it dissolves well in solvents like methanol and DMSO. Mirdametinib's crystalline form facilitates its formulation into oral medications, and its favorable permeability supports systemic absorption[6,7].

The drug undergoes hepatic metabolism and has a half-life that supports once-daily dosing. These physicochemical features contribute to its effective pharmacokinetic profile and therapeutic potential in MEK-driven malignancies[8,9]. The present study is the first to address this unmet need, aligning with ICH guidelines for method validation and forced degradation studies.

MATERIALS AND METHODOLOGY

Reagents and Chemicals

HPLC-grade acetonitrile (Merck), Milli-Q water (in-house), and AR-grade Formic acid and Tri fluoro acetic acid (Merck) were used for mobile phase preparation. The Mirdametinibreference standard was purchased from MedChemExpress, USA, and was used without further purification. All chemicals conformed to analytical quality standards.

Instruments

A Waters Alliance HPLC system operating in isocratic mode was employed for chromatographic separation. Complementary instruments used were a Eutech pH700 pH meter, a precision Sartorius BSA224S-CW balance, a UV-1700 spectrophotometer from Shimadzu, and a UCA 701 ultrasonicator from Unichrome.

Standard and Sample Solutions

Mirdametinib standard (10 mg) was sonicated in diluent and diluted to 10 mL (stock); 1 mL of stock was diluted to 10 mL for a 100 ppm working solution. The sample (47 mg) was similarly dissolved with 30 minutes of sonication, diluted to volume, and filtered to prepare a 100 ppm test solution.

Method Development

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Table 1: Evaluation of System Suitability for Mirdametinib

Parameter	Mirdametinib
Retention time	2.423
Plate count	7512
Tailing factor	1.05
% RSD	0.14

A series of chromatographic trials were conducted to optimize conditions for Mirdametinib analysis. Trials 1–3 using Waters X-Terra RP-18 and varying mobile phases showed issues like poor system suitability, excessive tailing, and unstable baseline. Trials 4 and 5 with Agilent Eclipse XDB improved resolution, but unknown peaks and inadequate peak height were observed. These trials highlighted the need for precise control of column type, mobile phase composition, and detection settings.

Method Validation

The RP-HPLC method established for Mirdametinib underwent validation following the protocols defined by ICH Q2(R1) standards[10-12].

System Suitability

Prior to initiating the method validation process, system suitability testing was carried out to confirm the reliability and consistency of the chromatographic system. Six successive injections of standard Mirdametinib were used to assess retention time, plate count, tailing factor, and %RSD, ensuring system performance and consistency.

Specificity

The specificity of the proposed HPLC method was established by analyzing blank, placebo, and standard drug solutions independently. These injections ensured there was no interference from excipients or matrix components at the retention time of Mirdametinib (2.423 min). The absence of overlapping or co-eluting peaks confirmed the method's ability to selectively quantify the analyte in the presence of other constituents.

Precision

System precision was assessed by injecting the same standard solution six times and determining the %RSD of peak areas to confirm consistency. Method precision involved preparing six separate sample solutions from the same batch and analyzing them under identical conditions. Intermediate precision was tested on a different day using a different analyst and HPLC system, confirming reproducibility across varying conditions[13].

Linearity

A linear calibration range from 25 to 150 µg/mL was established for Mirdametinib by analyzing standard solutions at six different concentrations. Triplicate readings were used to ensure accuracy, and a regression line was plotted. The correlation coefficient of above 0.999 confirmed that the method demonstrated excellent linearity throughout the evaluated range.

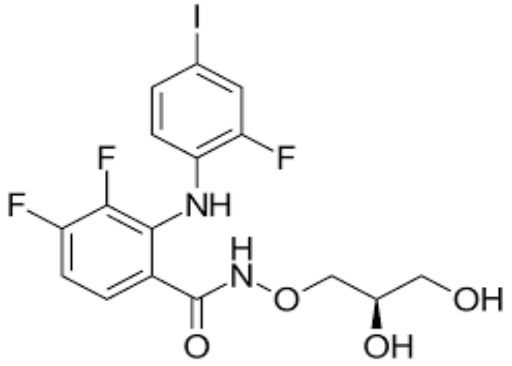

Figure 1: Chemical Structure of Mirdametinib

Table 2: Precision Evaluation of Mirdametinib by HPLC

S. No.	Conc. (µg/ml)	Area of Mirdametinib		
		System	Method	Intermediate
1.	100	2731345	2745163	2716150
2.		2735260	2732115	2727342
3.		2732104	2708964	2751679
4.		2727893	2770166	2730313
5.		2730558	2727580	2741228
6.		2738841	2751517	2709465
Mean	2732667		2739251	2729363
S.D	3850.860		21192.361	15583.856
% RSD	0.14		0.77	0.57

Table 3: HPLC Linearity Data for Mirdametinib

Mirdametinib	Conc. (µg/ml)	Peak area
	25.00	681977
	50.00	1361242
	75.00	2132553
	100.00	2734155
	125.00	3426189
	150.00	4150564
Regression equation	y = 27590.04x+272.61	
Slope	27590.04	
Intercept	272.61	
R^2	0.99979	

Accuracy

To verify the method's accuracy, known quantities of Mirdametinib were added to previously analyzed sample solutions at three concentration levels: 80%, 100%, and 120% of the target concentration. Each level was tested in triplicate using the established chromatographic conditions, assessing the method's capability to precisely quantify the drug in the presence of excipients[14].

Robustness and Sensitivity

Robustness was assessed by intentionally varying the flow rate (±0.1 mL/min) and organic content of the mobile phase (±5%). The method consistently maintained acceptable retention time, peak shape, and system suitability, demonstrating its reliability under slight operational changes. LOD and LOQ were calculated per ICH guidelines using response standard deviation and calibration curve slope, confirming the method's sensitivity for detecting low levels of Inavolisib[14,15].

Forced Degradation Studies

Forced degradation studies of Mirdametinib were conducted in accordance with ICH guidelines to evaluate the stability-indicating power of the developed HPLC method. The drug was subjected to stress conditions such as acidic, alkaline, oxidative, thermal, hydrolytic, and photolytic environments. After appropriate exposure durations, the samples were neutralized when required and analysed[16,17]. This helped in assessing the method's capability to separate Mirdametinib from its potential

Table 4: Accuracy results of Mirdametinib

Conc.	Area	% Recovery	Mean %Recovery
80%	4910235	99.8	100.1
	4937241	100.4	
	4921228	100.1	
100%	5461237	100.0	100.0
	5452338	99.8	
	5475896	100.2	
120%	6012345	100.0	100.3
	6041513	100.5	
	6028669	100.3	

Table 5: Robustness results of Mirdametinib

	Condition	Peak area	Tailing	% RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	2615342	1.13	0.45
	Actual flow (1.0ml)	2731345	1.05	0.14
	More flow (1.1ml)	2931635	1.03	0.41
Organic Phase change	Less Org (27:73)	2522461	1.16	0.65
	Actual (30:70)	2735260	1.07	0.14
	More Org (33:67)	3061264	0.99	0.55

RESULTS AND DISCUSSIONS

Method Optimization

The optimized chromatographic conditions involved the use of a Waters Alliance e-2695 HPLC system with an Agilent Eclipse XDB column (150 × 4.6 mm, 3.5 µm) under isocratic elution (Fig 2). The mobile phase consisted of acetonitrile and 0.1% formic acid in a 30:70 ratio. The detection was carried out at 223 nm, with an injection volume of 10 µL, a flow rate of 1 mL/min, and ambient temperature (25 °C).

System Suitability

The system suitability for Mirdametinib was established by evaluating key chromatographic parameters. The retention time was observed at 2.423 min, with a plate count of 7512 indicating good column efficiency. The tailing factor (1.05) and %RSD (0.14) were well within ICH-specified limits, confirming method suitability (Table 1).

Specificity

The blank chromatogram showed no peaks, and the placebo exhibited no signals overlapping with the drug's retention time at 2.423 minutes (as depicted in Fig. 3 and Fig. 4), indicating the absence of interference from the sample matrix. These findings confirmed that the method is highly specific, enabling reliable and accurate quantification of Mirdametinib without any contribution from endogenous substances or formulation excipients.

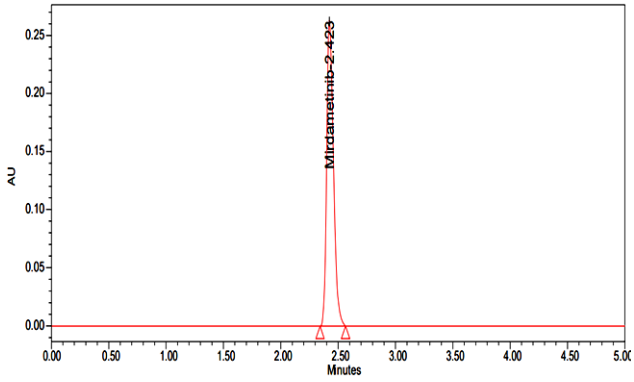

Figure 2: Optimized chromatogram of Mirdametinib

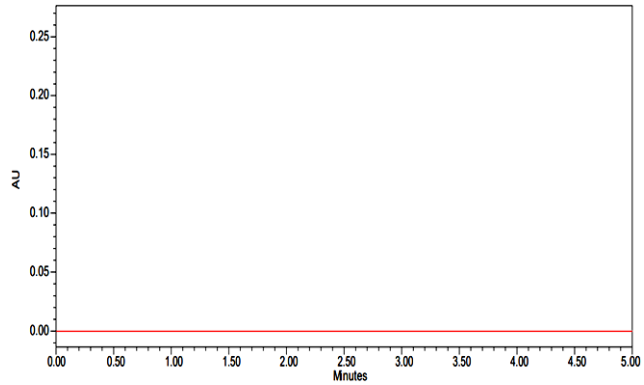

Figure 3: Blank Chromatogram

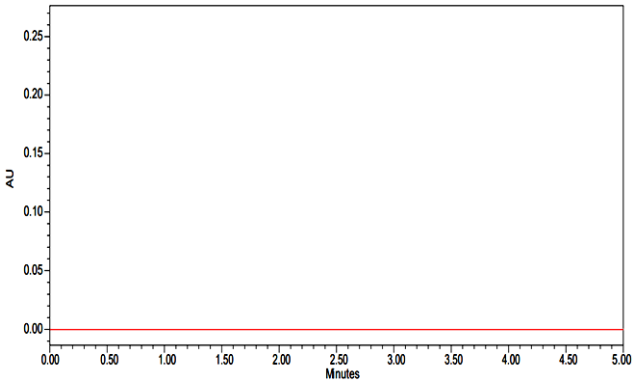

Figure 4: Placebo Sample Chromatogram

Precision

The precision studies including system, method, and intermediate precision showed %RSD values of 0.14, 0.77, and 0.57 respectively, all well within the acceptable limit of ≤2.0, confirming the reproducibility and consistency of the method (Table 2).

Linearity

The developed method demonstrated outstanding linearity for Mirdametinib across a wide concentration range of 25 to 150 µg/mL. The R^2 was found to be 0.99967, reflecting a highly linear response between concentration and peak area. This strong linearity confirms the method's suitability for accurate and reliable quantification of Mirdametinib in both lower and higher concentration levels, ensuring consistent analytical performance as illustrated in Table 3 and Figure 5.

Accuracy

Accuracy studies for Mirdametinib showed mean recovery values ranging from 100.0 to 100.3 % at 80%, 100%, and 120% concentration levels, demonstrating the method's reliability and reproducibility (Table 4).

Assay, LOD and LOQ

The assay of Mirdametinib in the marketed formulation (GOMEKLI) demonstrated a percent assay of 99.8%, indicating excellent accuracy and label claim compliance. Mirdametinib showed an LOD of 0.60 µg/mL and an LOQ of 2.0 µg/mL.

Table 6: Stress Degradation Study Outcomes for Mirdametinib

Degra. Conditions	Area	% Assay	% Degra.
Control	2731564	100	0
Acid	2393654	87.6	12.4
Alkali	2426147	88.8	11.2
Peroxide	2345709	85.9	14.1
Reduction	2704615	99.0	1.0
Thermal	2480792	90.8	9.2
Photolytic	2640238	96.7	3.3
Hydrolysis	2617279	95.8	4.2

Robustness

The robustness study of Mirdametinib confirmed that minor deliberate changes in flow rate and organic phase composition did not significantly affect the method performance. All parameters remained within acceptable limits, as shown in Table 5.

Forced Degradation Studies

Forced degradation studies of Mirdametinib were conducted under various stress conditions to assess the stability-indicating nature of the developed method. The control sample showed 100% assay with no degradation. Acidic and alkaline hydrolysis resulted in 12.4% and 11.2% degradation, respectively, while oxidative stress (peroxide) led to the highest degradation of 14.1%. Thermal degradation showed a moderate effect with 9.2% degradation. Photolytic and hydrolytic conditions caused 3.3% and 4.2% degradation, respectively, indicating some susceptibility under light and aqueous environments. Reduction stress had minimal impact, showing only 1.0% degradation. These results confirm the method's capability to effectively separate the drug from its degradation products in Table 6.

CONCLUSION

The present study describes a robust, stability-indicating RP-HPLC method developed and validated for the estimation of Mirdametinib in bulk and dosage forms. Utilizing a simple isocratic elution with readily available solvents and a short run time of 5 minutes, the method proves to be both efficient and cost-effective. The validation results confirm its compliance with ICH Q2(R1) guidelines, ensuring excellent specificity, precision, linearity, accuracy, robustness, and sensitivity. Notably, forced degradation studies under various stress conditions confirmed the method's stability-indicating nature by effectively separating the drug from its degradation products. The successful application of the method to a commercial formulation highlights its suitability for routine analysis and quality control in pharmaceutical industries. Given its accuracy, reproducibility, and ability to detect low concentrations, the proposed RP-HPLC method stands as a valuable tool in the analytical evaluation and long-term stability monitoring of Mirdametinib formulations.

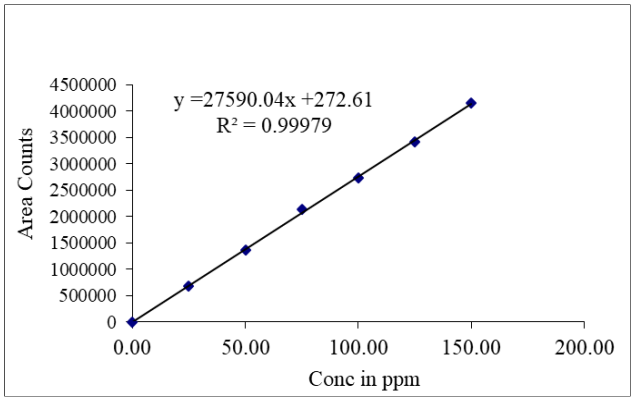

Figure 5: Calibration curve for Mirdametinib at 223 nm

REFERENCES

1. Yeh TC, Marsh V, Bernat BA, Ballard J, Colwell H, Evans RJ, et al. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. Cancer Res. 2007;67(5):2170–9.
2. Sullivan RJ, Infante JR, Janku F, Wong DJL, Sosman JA, Keedy VL, et al. First-in-class MEK1/2 inhibitor Mirdametinib (PD-0325901) in advanced solid tumors: results of a phase I dose-escalation study. Clin Cancer Res. *2018*;24(12):3010–9.
3. Li J, Zheng S, Chen B, Butte AJ, Swamidass SJ, Lu Z. A survey of current trends in computational drug repositioning. Brief Bioinform. 2016;17(1):2–12.
4. Avila C, Horiuchi KY, Farrell L, Schlessinger J, Bence KK. PD-0325901: A MEK inhibitor with high oral bioavailability and potential utility in treatment of RAS/RAF-mutant tumors. J Med Chem. 2006;49(20):6589–607.
5. Vikhar A, Ahmad D, Shaikh MS, Yasar Q. Unexplored Enigma in Flemingins against Cholangiocarcinoma : A Network Pharmacology and Molecular Docking Approach to Predict Molecular Mechanisms. Chem Methodol. 2025;9:610–30.
6. Singh J, Petter RC, Baillie TA, Whitty A. The resurgence of covalent drugs. Nat Rev Drug Discov. 2011;10(4):307–17.
7. European Medicines Agency (EMA). Mirdametinib: Scientific Discussion and Assessment Report. EMA/CHMP/2021.
8. U.S. Food and Drug Administration. Investigational New Drug Application: PD-0325901. FDA IND File No. 2020.
9. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer*. 2009;9(1):28–39.
10. Chandramore KR, Sonawane SS, Ahire RS, Reddy H, Ahire SB, Jadhav PB, et al. Development and Validation of Stability Indicating LC Method for Selexipag : In-Silico Toxicity Study and Characterization of its Degradation Products. Chem Methodol. 2025;9:427–47.
11. Konatham TK, Anuradha M, Narmada A. a stability indicating method development and validation of Telmisartan and Nifedipine in pure form using RP-HPLC. International Journal of Pharmaceutical, Biological and Chemical Sciences. 2020;9(3):36-44.
12. Yelampalli SR, Gandla K, Reddy KTK, Ibrahim AE, El Deeb S. Determination of Sodium, Potassium, and Magnesium as Sulfate Salts in Oral Preparations Using

Ion Chromatography and Conductivity Detection. Separations. 2023; 10(2):99.
13. Nagda D, Captain A. Validated Green Densitometric Method for Estimation of Naringin Biomarker in Hydroalcoholic Extract of Leaves of Ceiba Pentandra (L .) Gaertn. Asian J Green Chem. 2025;9:427–42.
14. Reddy KTK, Haque MA. Development and Validation of Aducanumab by Bioanalytical Method Using Liquid Chromatography-Tandem Mass Spectroscopy. Adv J Chem Sect A. 2025;8(3):456–68.
15. Mohammadzadeh Jahani P, Zaimbashi R, Beitollahi H, Tajik S. An Electrochemical Sensor Based on SnSe nanosheets and ionic liquids for the Selective Detection of N-acetylcysteine. Chem Methodol. 2025;9(3):158–71.
16. Preeti N. Yadav, Chhalotiya Usmangani K, Patel Kesha M, Tandel Jinal N. Quantification of A β Adrenergic Receptor Drug Mirabegron by Stability Indicating LC Method andUv–visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form. Chem Methodol [Internet]. 2020;4(53):340–58. Available from: http://chemmethod.com
17. Elumalai S, Sharma M, Dantinapalli VLS, Palanisamy M. Novel Stability Indicating UPLC Method Development and Validation for Simultaneous Quantification of Perindopril Arginine and Bisoprolol Fumarate in Pure and Pharmaceutical Dosage Form. Adv J Chem Sect A. 2025;8(9):1488–507.