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Development of a Novel and Robust HPTLC Method for the Assessment of Ivosidenib in Solid Dosage Form with Analytical Greenness Evaluation

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ABSTRACT

A novel, efficient, and rapid high-performance thin layer chromatographic (HPTLC) technique has been developed and validated for the quantitative analysis of Ivosidenib in both bulk and formulated products. The chromatographic process utilizes HPTLC plates coated with silica gel 60 F254, employing a mobile phase composed of Hexane, ethyl acetate, ethanol, and glacial acetic acid in a ratio of 6:2:2:0.02 (v/v/v/v). Detection was performed densitometrically at a wavelength of 245 nm, yielding an Rf value for the drug of 0.62. Validation of the method was conducted in accordance with ICH guidelines, focusing on parameters such as linearity, accuracy, precision, and robustness. The calibration curve exhibited linearity across a range of 50-250 ng/band, with a regression coefficient of 0.999. The method demonstrated a high accuracy rate of 97.84%, and the %RSD values for both intra-day and inter-day variations did not exceed 2.0. This method showcases exceptional sensitivity and specificity, making it a new, straightforward, and cost-effective approach for the routine estimation of Ivosidenib in bulk and pharmaceutical formulations, thereby assisting both industries and researchers in the rapid and economical determination of Ivosidenib in standard analyses. The AGREE software is a metric system designed to evaluate the analytical greenness of methods according to significance principles. The proposed method achieved a score of 0.62 when assessed using AGREE.

Keywords: HPTLC, Ivosidenib, validation, Development, analysis, Agree Score

INTRODUCTION

Ivosidenib, also referred to as AG-12, is an antineoplastic drug used for the treatment of acute myeloid leukemia and cholangiocarcinoma in adults who have a susceptible IDH1 mutation. It functions by inhibiting the mutant IDH (isocitrate dehydrogenase) enzyme. Ivosidenib received FDA approval on May 2, 2019, and is developed by Angios Pharmaceuticals. It is marketed under the brand name Tibsovo (Ivosidenib 250 mg) [(1)] Ivosidenib functions by targeting the mutant IDH1 enzyme, which is a metabolic enzyme responsible for converting isocitrate into alpha-ketoglutarate to generate NADPH. In its mutated form, IDH1 instead converts isocitrate into D-2-hydroxyglutarate, an oncometabolite. Ivosidenib is a small-molecule inhibitor specifically designed to block the activity of the mutant IDH1 enzyme, particularly the IDH1-R132 variant. By binding to this mutant enzyme, ivosidenib effectively halts the conversion of alpha-ketoglutarate to D-2-hydroxyglutarate, resulting in decreased levels of D-2-hydroxyglutarate within the cell. (Lavacchi et al., 2021)

A review of the literature indicates that there is currently no official HPTLC method documented for the quantification of ivosidenib in formulations within any pharmacopoeias such as IP, USP, BP, or EP, nor is there a published method available. Some analytical methods that have been reported pertain to the estimation of ivosidenib in human and rat plasma for pharmacokinetic studies, along with one HPLC method for its quantification in formulations. (Chilaka Ratnaraju & Dr. Raviteja Bandla, 2025) reported HPLC approach for the quantification of ivosidenib in bulk drug and pharmaceutical formulations. (Gando & Yasu, 2023) described an HPLC-UV method for determining ivosidenib in human plasma. (Chen et al. 2023) reported a UPLC-MS/MS method for the determination of ivosidenib in rat plasma and (Dittakavi et al. 2019) reported an LC-ESI-MS/MS method for the determination of ivosidenib in 10µL of mouse plasma.

HPTLC is utilized for both qualitative and quantitative analytical purposes, including the analysis of herbal and dietary supplements, nutraceuticals, and a variety of medications. It plays a crucial role in routine quality control and purity assessments, as well as in clinical applications such as metabolism studies and drug screening. Additionally, it is employed in forensic investigations, including poisoning cases, the assessment of radiochemical impurities in radiopharmaceuticals, and the detection and identification of pharmaceutical raw materials, drugs, and their metabolites in biological samples. Benefits of HPTLC compared to HPLC include the absence of costly columns that require meticulous upkeep for analyte separation. HPTLC utilizes less solvent for analysis, allows

for the simultaneous analysis of multiple samples on a single TLC plate, thereby saving time and enhancing sample throughput. Additionally, the visual detection of spots on TLC plates aids in qualitative analysis. (Partha Sarathi Bairy, 2015).

The objective of the present study was to develop HPTLC method for estimation of Ivosidenib, to validate the developed HPTLC method as per ICH guidelines, to apply the developed HPTLC method for analysis of marketed formulation containing the above stated drug.

Materials

Reagents and chemicals: Ivosidenib was obtained as a gift sample from CDTL Mumbai. All solvents used were of analytical grade. Ethyl Acetate procured from Hyma Synthesis Pvt. Ltd; Acetic Acid Glacial procured from molychem, Hexane and Ethanol were procured from SRL Pvt. Ltd.

Equipment: Accuratio, FSST-503 of Mettler toledo Weighing balance, borosil pipette, HPTLC system (Camag, Switzerland) consisted of a CAMAG- LINOMAT V automatic sample applicator, Linomet Micro syringe, CAMAG UV Cabinet with dual wavelength UV lamp and densitometric analysis were carried out using a camag TLC scanner with Software VisionCATS, TLC plate Silica gel 60 F_{254} aluminium sheets (20*20cm, layer thickness 0.2mm) (E. Merck LGaA, Darmstadt, Germany) was used for the present study.

Preparation

Preparation of standard solution: Ivosidenib stock solution (100 µg/mL) was prepared by dissolving in an ethanol as a diluent. Then, pipette out 5ml from the above stock solution diluted up to 10ml with diluent to make (50 µg/ml) solution.

Preparation of sample solution:

Dissolve one tablet (equivalent to 250 mg) in ethanol and bring the volume to 100 mL in a volumetric flask (2500 µg/mL). Then, pipette 1 mL of this solution into a 50 mL volumetric flask and bring the volume to 50 mL with diluent (50 µg/mL).

Preparation of mobile phase:

A mixture of (Hexane: ethyl acetate: ethanol: Glacial acetic acid) was prepared in the ratio of (6:2:2:0.02) (v/v/v/v) in a twin trough chamber.

Chromatographic conditions

A total of 4 µl of both the standard and sample solutions were applied to the TLC plate utilizing a Camage Linomet V automatic sample applicator, forming bands with a bandwidth of 8 mm and a distance of 11.4 mm between each band, using a microsyringe. The application rate was maintained at a constant 150 nL/s. Following application, the plate was allowed to dry for 5 minutes at ambient temperature and subsequently saturated for 20 minutes in a twin trough glass chamber containing the mobile phase. The plate was then immersed in the mobile phase, and ascending development was conducted to a distance of 7 cm. After development, the plates were air-dried, and densitometric scanning was executed at a wavelength of 245 nm using a Camage TLC scanner. The Retardation Factor (Rf) was determined to be 0.6 ± 0.02. The radiation source utilized was a deuterium lamp that emitted a continuous UV spectrum ranging from 200 to 300 nm. The densitogram derived from a standard solution of ivosidenib is illustrated in Figure 1.

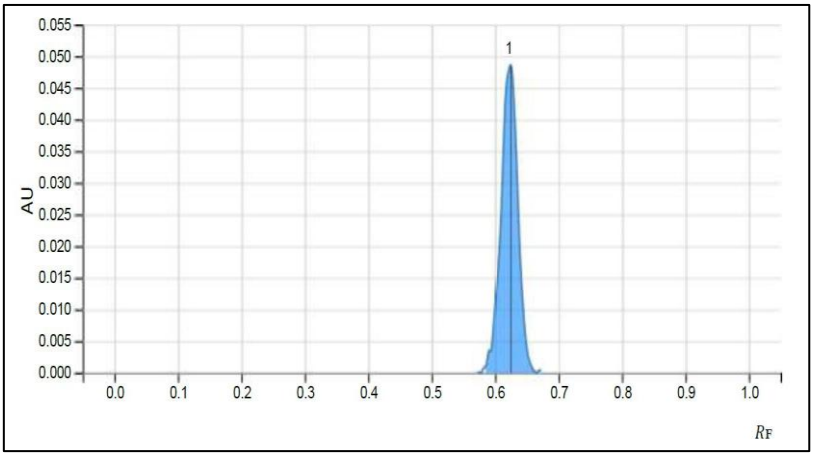

Figure 1: HPTLC densitogram of Standard solution.

Validation of the developed method [9,10]

The validation of the HPTLC method was carried out in accordance with ICH guidelines [ICH Q2(R1)] with respect to the following parameters (Kumari Jaiswal & Jain, 2022).

Specificity

Evaluates the capability of the HPTLC method to accurately quantify the target analyte(s) while avoiding interference from other substances in the sample matrix. The method's specificity was established by examining both the standard drug and the test sample. The presence of ivosidenib in the sample and standard, along with the diluent and mobile phase, was verified by comparing their Rf values.

Linearity

The linearity of an analytical method denotes its ability to produce test results that are directly proportional to the concentration of the analyte within a defined range.

The linearity of the method was assessed by creating calibration curves at five distinct concentration levels. These curves were generated across a concentration range of 50 to 250 ng/band. Standard working solutions of 1, 2, 3, 4, and 5 µL/band were applied to the plate, resulting in concentrations of 50, 100, 150, 200, and 250 ng/band of ivosidenib, with scanning performed at a wavelength of 245 nm.

The calibration curve was constructed by plotting the peak area against the concentrations.

Accuracy

To verify the Accuracy of the analytical method, recovery tests were conducted by introducing a standard drug solution at three distinct concentrations (80%, 100%, and 120%) into a pre-analyzed sample solution (200 ng/band), ensuring that the samples remained within the linear range after the addition of standards. Each concentration level was assessed through three replicate measurements.

Precision

The precision of an analytical technique reflects the reliability of measurements acquired from repeatedly testing the identical sample under regulated conditions. Precision can be assessed through repeatability, intermediate precision, and reproducibility. Typically, the precision of an analytical method is represented by the variance, standard deviation, or coefficient of variation derived from a set of measurements. (ICH Q2 R2).

a) **Repeatability** refers to the consistency of measurements taken under identical conditions within a brief time frame (for instance, within a day, with three measurements spaced two hours apart). It is also known as intra-assay precision.

To evaluate repeatability, at least nine measurements should be conducted across the reportable range of the procedure (for example, three concentrations with three replicates each).

b) **Intermediate precision**, also known as inter-day precision, refers to the variations within laboratories that occur due to factors such as different days, environmental conditions, analysts, and equipment.

Robustness

Robustness evaluates the method's capacity to remain stable despite minor, intentional changes in parameters like mobile phase composition, chamber saturation time, and mobile phase volume, and the impact of these variations on the results was analyzed.

a) **Changes in saturation duration:** The reliability of the method was assessed in triplicate at a concentration of 200ng/band, for both standard and sample at intervals of 10, 20, and 30 minutes. The average and %RSD of the peak area were computed.

b) **Changes in the composition of the mobile phase:** The method's reliability was evaluated in triplicate at a concentration of 200 ng/band for both the standard and the sample. The average and percentage relative standard deviation (%RSD) of the peak area were calculated.

c) **Changes in the mobile phase volume:** The method's reliability was assessed in triplicate at a concentration of 200 ng/band for both the standard and the sample volumes of 8ml, 10ml, and 12ml. The average and percentage relative standard deviation (%RSD) of the peak area were determined.

Results and Discussion

1) **Specificity:** The presence of the band for Ivosidenib in the sample was validated by comparing its Rf value and spectrum to those of a standard. No peaks were detected in the bands of the diluent and mobile phase, indicating the absence of interference and confirming the method's specificity in the presence of different excipients.

Table 1: Result for Specificity

S.NO	Description	Vol. (µL)	ng/band	Rf
1.	Ivosidenib 0.05mg/ml in Ethanol (Standarad)	4.0	200	0.62
2.	Ivosidenib 0.05mg/ml in Ethanol (Sample)	4.0	200	0.61
3.	Mobile Phase	4.0	--	0.02
4.	Diluent (Ethanol)	4.0	--	0.01

2) **Linearity:** A linear correlation was established by graphing different concentrations of ivosidenib against their respective peak areas, indicating a linear response across the concentration range of 50-250 ng/band. A graph was constructed with concentration represented on the X-axis and peak area on the Y-axis. The drug showed a robust correlation, achieving a coefficient of determination (R^2) of 0.999.

Table 2: Result for Linearity study

Vol. (µL)	ng/band	Rf	Area
1.	50	0.61	0.00033
2.	100	0.6	0.00077
3.	150	0.62	0.00119
4.	200	0.59	0.00157
5.	250	0.61	0.00199

Table 2.1: Linear regression obtained from Calibration Curve

Range (ng/band)	R^2	CV	Slope	Intercept
50-250	0.999	1.09%	0.00000824	-0.0000660

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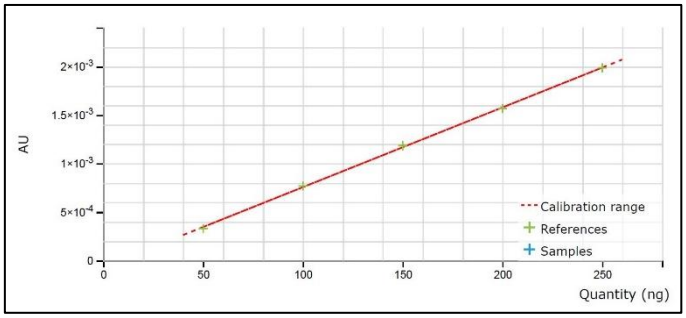

Figure 2: Calibration plot for Ivosidenib

Table 3: Result of Accuracy study

Level	Volume (µL) (Sample + Standard)	ng/band (Sample + Standard)	Area	Average Area	Expected Area	S.D	% RSD	% Recovery	Mean % Recovery
80%	4 + 4	360	0.00278	0.00273	0.00283	0.00004	1.38	96.59	97.29
			0.00271						
			0.00272						
100%	4 + 4	400	0.00295	0.00299	0.00307	0.00005	1.71	97.40	
			0.00298						
			0.00305						
120%	4 + 4	440	0.00291	0.00295	0.00301	0.00004	1.36	97.90	
			0.00299						
			0.00295						

3) **Accuracy:** The optimized proposed method demonstrated % recovery is within acceptable range which is 95% to 105% and %RSD is within the limit.

4) **Precision:**

a) **Intermediate Precision:** The %RSD values of 1.01%, 1.06%, 1.19%, 1.16% of Ivosidenib in interday analysis were within the acceptable limit which is less than 2% at different concentration as given in the below table. Hence, the method was found to be precise. Each concentration is replicated thrice.

Table 4.3: Outcome of Day 3

Table 4.1: Outcome of Day 1

Con.(ng/band)	Avg. Area
100	0.00069
150	0.00109
200	0.00146
250	0.00181

Table 4.2: Outcome of Day 2

Con.(ng/band)	Avg. Area
100	0.00069
150	0.00112
200	0.00147
250	0.00181

Table 4.3: Outcome of Day 3

Con.(ng/band)	Avg. Area
100	0.00068
150	0.00110
200	0.0015
250	0.00185

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Table 4: Result of intermediate precision

Conc. ng/band	**Average Area of different days**	**SD**	**% RSD**
100	0.000687778	6.9389E-06	1.01
150	0.001107778	1.17063E-05	1.06
200	0.00148	1.76383E-05	1.19
250	0.001825556	2.11695E-05	1.16

b) **Intraday precision (Repeatability):** The %RSD values of 1.05%, 1.08%, 1.14%, 1.01% of Ivosidenib in intraday analysis were within the acceptable limit which is less than 2% at different concentration as given in the below table. Hence, method was found to be precise. Each concentration is replicated thrice.

Table 5.1: Result of 10:00 AM

Con.(ng/band)	Avg. Area
100	0.000686
150	0.00110
200	0.00145
250	0.00180

Table 5.2: Result of 01:00 PM

Con.(ng/band)	Avg. Area
100	0.000673
150	0.00113
200	0.00147
250	0.00181

Table 5.3: Result of 04:00 PM

Con.(ng/band)	Avg. Area
100	0.000675
150	0.00111
200	0.00148
250	0.00184

Table 5: Result of intraday precision

Conc. ng/band	Average Area at different time intervals	SD	% RSD
100	0.000678556	7.12065E-06	1.05
150	0.001116667	1.20185E-05	1.08
200	0.001471111	1.67774E-05	1.14
250	0.001818889	1.83586E-05	1.01

5) **Robustness:** The method demonstrated robustness, yielding consistent results characterized by low % RSD and area values. Additionally, the outcomes were not influenced by intentional modifications to the parameters.

Table 6: Result for Robustness

Parameter	Parameter Changed	Peak Area		Average Area		Standard Deviation		% RSD	
		Std.	Sample	Std.	Sample	reference	Sample	Std.	Sample
Changed in Mobile phase volume	8 ml	0.0019	0.001837	0.00201	0.00182	2.160E-05	2.114E-05	1.07	1.16
	10 ml	0.0021	0.001787						
	12 ml	0.0021	0.001823						
Change in Saturation Time	10 min	0.0033	0.0016	0.00332	0.00165	3.715E-05	1.771E-05	1.12	1.07
	20 min	0.0033	0.0016						
	30 min	0.0034	0.0017						
Changed in Mobile phase Ratio (H:E:ET:GA)	5:3:2:0.02 (v/v/v/v)	0.0034	0.00163	0.00336	0.00164	3.871E-05	1.812E-05	1.15	1.10
	6:2:2:0.02 (v/v/v/v)	0.0036	0.00167						
	7:1:2:0.02 (v/v/v/v)	0.0035	0.00163						

H: Hexane, E: Ethyl acetate, ET: Ethanol, GA: Glacial Acetic acid

Std.=Standard solution/Reference solution. Sample= Sample solution

Assessment of Analytical Greenness using AGREE software: Green analytical chemistry aims to enhance the environmental sustainability and safety of analytical methods for humans. The Analytical Greenness calculator is a versatile, user-centric evaluation tool that provides clear and insightful results. Its assessment criteria are based on the 12 principles of green analytical chemistry, which are translated into a standardized scale of 0 to 1. The ultimate score is calculated according to the SIGNIFICANCE principles, producing a pictogram that illustrates the overall score, the analytical method's performance for each criterion, and the weights designated by the use.

All 12 input variables are standardized to a common scale ranging from 0 to 1. The result is represented as a clock-like graph, featuring an overall score and color coding at the center (refer to Figure 3. The effectiveness of the procedure for each principle is illustrated using an intuitive red-yellow-green color scale, while the significance of each principle is indicated by the width of its respective segments. Users can easily perform the assessment with user-friendly software that generates both a graph and an assessment report automatically. The total score, shown prominently in the center of the pictogram, ranges from 0.5 to 1, with a light green color suggesting that the evaluated procedure requires adjustments in the mobile phase selection to enhance its environmental friendliness. Each assessment criterion's performance is represented by the color of the corresponding segment, with numbers assigned to each criterion.

Direct analytical methods must be utilized to prevent sample treatment. Measurements should be conducted in situ with minimal sample size. The preparation process should be streamlined, emphasizing automation while avoiding derivation and minimizing generated waste. Preference should be given to multi-analyte or multi-parameter techniques, and the use of high-energy instruments should be avoided. Reagents sourced from renewable materials are recommended, and the elimination of toxic substances is essential to enhance operator safety. (Pena-Pereira et al., 2022)

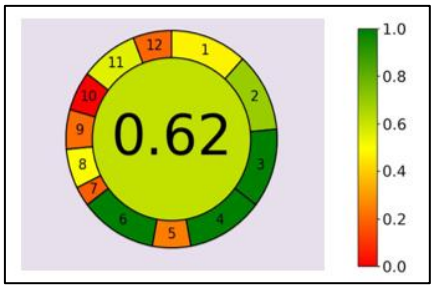

Figure 3: The environmental sustainability of the method developed with AGREE software. featuring a general assessment result on the left and the associated color scale for reference on the right.

Conclusion: This research successfully established and validated a novel, straightforward, precise, and accurate High-Performance Thin-Layer Chromatography (HPTLC) technique for the qualitative and quantitative analysis of Ivosidenib in solid dosage form. The validation adhered to ICH guidelines, evaluating essential parameters such as system suitability, specificity, precision, accuracy, robustness, limits of detection (LOD), and limits of quantification (LOQ). This represents the first documented HPTLC method for Ivosidenib, featuring minimal sample preparation, shortened analysis time, reduced solvent usage, and cost efficiency, making it ideal for routine analysis and quality control of Ivosidenib in tablet forms. The chromatographic analysis utilized an optimized mobile phase composed of hexane, ethyl acetate, ethanol, and glacial acetic acid. Separation was accomplished on aluminum plates coated with silica gel 60 F_{254}, with the drug peak distinctly resolved and detected using a UV detector at 245 nm. The AGREE software evaluated the environmental friendliness score of the proposed method, which was found to be 0.62.

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Conflict of Interest Statement: All authors have nothing else to disclose.