IJMPR, 2026, 10(5), 67-73 ISSN: 2319-5878

INTERNATIONAL JOURNAL OF MODERN PHARMACEUTICAL RESEARCH

www.ijmpronline.com

Research Article

Impact Factor: 7.533

Coden USA: IJMPR3

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IVOSIDENIB IMPURITY 1

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Article Received on: 04/04/2026 **Article Revised on: 25/04/2026** **Article Published on: 01/05/2026**

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https://doi.org/10.5281/zenodo.19884264

How to cite this Article: Parmar Aditi[1], Dr. Bhumi R. Patel[2*], Dr. Jaymin G. Patel[2], Mr. Ronak N. Patel[2], Ms. Pooja Soni[3], Dr. Divyakant Patel[4]. (2026). Rp-Hplc Method Development And Validation For Estimation of Ivosidenib Impurity 1. International Journal of Modern Pharmaceutical Research, 10(5), 67–73.

ABSTRACT

A Novel, simple, precise, specific, accurate and rapid Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method for estimation of Ivosidenib Impurity 1, Ivosidenib API has been developed and validated. The separation was achieved by employing column Agilent zorbax C18 (250 mm × 4.6 mm × 5 µm) as a stationary phase, In gradient program mixture of Mobile phase A: 0.1% Formic acid: methanol: IPA:GAA(60:35:5:0.5v/v/v/v) Mobile phase B: Phosphate buffer pH 5 at a flow rate of 1.0 mL/min. Detection wavelength was kept at 237 nm, 248 nm using PDA detector, temperature was kept at 25°C. Retention time was noted to be 7.378 min, 19.690 min for Ivosidenib impurity 1, Ivosidenib API respectively. The various analytical validation parameters, including specificity, linearity, LOD, LOQ, precision, accuracy, and robustness were determined as per ICH Q2(R2) guidelines. The method was linear over the range of 50-100 µg/ml, 200-600 µg/ml, for Ivosidenib impurity 1, Ivosidenib API respectively. Hence, the developed RP-HPLC method was found to be specific, accurate, precise, and robust for estimation of Ivosidenib Impurity 1 and Ivosidenib API.

KEYWORDS: RP-HPLC Method, Method Development, Analytical Method Validation (AMV), Ivosidenib Impurity 1, Ivosidenib API.

INTRODUCTION

Ivosidenib, commercially known as Tibsovo, is an anticancer agent indicated for the treatment of acute myeloid leukaemia (AML) and cholangiocarcinoma. Acute Myeloid Leukaemia (AML) is a rapidly malignancy originating in the bone marrow, a soft internal tissue of bones that is necessary for the formation and development of blood cells. In AML, patients have an excess of abnormal white blood cells that cannot fight infection. This makes infections more frequent. The disease also lowers the number of red blood cells, leading to anaemia, which causes fatigue, paleness, and shortness of breath. In addition, the reduction of platelets results in easy bleeding and bruising.[1,2] Cholangiocarcinoma, or bile duct cancer, is a rare form of malignancy arising from the epithelial lining of the biliary tract. The bile ducts function to convey bile, a liver-derived digestive secretion essential for lipid digestion.[3] Ivosidenib is a targeted cancer drug that works by inhibiting the mutant isocitrate dehydrogenase-1 (mIDH1) enzyme. IDH1 mutations lead to the production of an abnormal metabolite, 2-hydroxyglutarate (2-HG), which blocks normal cell differentiation and promotes cancer growth. By inhibiting mIDH1, ivosidenib lowers 2-HG levels, restores normal cell differentiation, and helps control cancer growth.[4,5,6] Consequently, monitoring and controlling impurities are important requirements outlined in regulatory guidelines such as ICH Q3A (R2)[7] and Q3B (R2).[8]

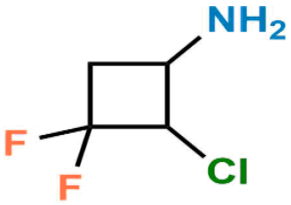

Fig 1 Structure of Ivosidenib Impurity – 1.

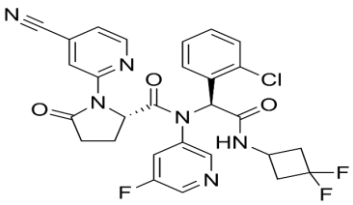

Fig 2 Structure of Ivosidenib API.

Literature on analytical method development and validation for Ivosidenib and its impurities is very limited. In particular, there is a lack of reported methods addressing the detection and quantification of Ivosidenib Impurity 1[9], so the present study aims to develop and validate a straightforward, precise, accurate, and robust RP-HPLC method for the estimation of Impurity 1 of Ivosidenib.

MATERIALS AND METHODS

Ivosidenib impurity -1 was obtained from cleanchem laboratories and Ivosidenib API was received as a gift sample. Other chemicals such as Methanol, Acetonitrile, Water, Formic acid, Isopropyl alcohol (IPA) were of analytical HPLC grade. Phosphate buffer (pH -5) HPLC grade was used in method development trial.

Instrument Details

Agilent 1100, HPLC Software (Clarityonline) with Detector PDA. Digital balance of Sartorius CP224S, pH meter, Melting point apparatus, Shimadzu IR Spectrophotometer were used.

Wavelength Selection

The Overlay spectra of both drugs were recorded in the UV region (200-400 nm). The API showed maximum absorbance at 248 nm and the impurity at 237 nm. Therefore, chromatographic monitoring was performed at 248 nm for API and 237 nm for impurity to achieve maximum sensitivity and adequate selectivity for each analyte using PDA detector.

Method Development

Various mobile phase types were investigated in the development method for impurity 1. The suitability of the mobile phase was decided on the basis of the selectivity and sensitivity of impurity and API.

Finally good separation were achieved by employing column Agilent zorbax C18 (250 mm × 4.6 mm × 5 µm) as a stationary phase, In gradient program mixture of Mobile phase A: 0.1% Formic acid: methanol: IPA:GAA(60:35:5:0.5v/v/v/v) Mobile phase B: Phosphate buffer pH 5 at a flow rate of 1.0 mL/min. Detection wavelength was kept at 237 nm, 248 nm using PDA detector, temperature was kept at 25°C. Retention time was noted to be 7.378 min, 19.690 min for Ivosidenib impurity 1, Ivosidenib API respectively.

Preparation of Mobile Phase

Mobile Phase A was prepared by mixing 0.1% (v/v) formic acid, methanol, isopropyl alcohol, and glacial acetic acid in the ratio of 60:35:5:0.5 (v/v/v/v). The components were mixed thoroughly to obtain a homogeneous solution. Mobile Phase B consisted of phosphate buffer pH 5.0.

The mobile phases were filtered and degassed before use. The optimized conditions showed improved resolution and acceptable peak shape.

A gradient elution mode was applied to enhance selectivity and obtain satisfactory separation between the impurity and API.

Preparation of 0.1% Formic acid: 0.1 ml formic acid in 100 ml water.

Preparation of Phosphate buffer pH 5.0: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 ml of water and adjusted to pH 5.0 with 10 M potassium hydroxide.

Preparation of 100 ppm standard solution of Ivosidenib Impurity-1:

An accurately weighed quantity of 10 mg of Ivosidenib Impurity 1 was transferred into a 100 mL volumetric flask. The sample was dissolved in approximately 15 mL of methanol with the aid of sonication, and the volume was then made up to the mark with methanol. The resulting solution was thoroughly mixed to obtain a final concentration of 100 ppm.

Preparation of 400 ppm standard solution of Ivosidenib API

An accurately weighed quantity of 40 mg of Ivosidenib API was transferred into a 100 mL volumetric flask. The sample was dissolved in approximately 15 mL of methanol with sonication, and the volume was then adjusted to 100 mL with methanol. The resulting solution was thoroughly mixed to obtain a final concentration of 400 ppm.

Mixture of standard solution of 100 ppm Ivosidenib impurity-1 and 400 ppm Ivosidenib API

An accurately weighed quantity of 10 mg of Impurity-1 and 40 mg of Ivosidenib API was transferred into a 100 mL volumetric flask. The contents were dissolved in a small volume of methanol with the aid of sonication to ensure complete dissolution. The solution was then

diluted to the mark with methanol and mixed thoroughly to obtain final concentrations of 100 ppm of Impurity-1 and 400 ppm of the API.

Method Validation[10]

1) Linearity and Range

The linearity for Ivosidenib Impurity 1 and Ivosidenib API were assessed by analysis of combined standard solution in range of 50-150 µg/ml and 200-600 µg/ml respectively. Correlation co-efficient for calibration curve Ivosidenib Impurity 1 and Ivosidenib API was found to be 0.99991 and 0.9999.

2) Precision

Precision was evaluated at three levels: intermediate precision (intraday precision), reproducibility (interday precision), and repeatability. The solution containing Ivosidenib Impurity 1(100 µg/ml) and Ivosidenib API (400 µg/ml) was injected six times for repeatability study. Intermediate precision study was performed by injecting 50,100,150 µg/ml of Ivosidenib impurity 1 and 200 400, 600 µg/ml of Ivosidenib API solutions three times for each aliquot. The %RSD for precision was calculated.

3) Limit of Detection and Limit of Quantitation

The LOD and LOQ were separately determined from calibration curve.

Then LOD and LOQ were calculated using following equation:

$$LOD = 3.3* \sigma/s \text{ and } LOQ = 10* \sigma/s$$

Where, σ = Standard deviation of Y-intercepts, S = Mean slope of calibration curve.

4) Accuracy

The accuracy of the method was assessed using the standard addition technique, where a known amount of the working standard was spiked into a mixture of drug at three concentration levels: 80%, 100%, and 120% of the standard concentration. Each solution was injected in triplicate and the recovery was calculated by measuring peak areas.

5) Robustness

The robustness of the analytical procedure was evaluated to assess its ability to remain unaffected by minor but deliberate variations in method parameters, ensuring its reliability during routine use. Robustness testing was conducted (n=3) by altering key parameters, including:

Flow rate of the mobile phase (± 0.2 ml/min)

Mobile phase (± 2 %)

ph (±0.2).

RESULT AND DISCUSSION

Table 1: Optimized Chromatographic Conditions.

<table>
<tr><td>PARAMETER</td><td>CHROMATOGRAPHIC CONDITIONS</td></tr>
<tr><td>Elution mode</td><td>Gradient</td></tr>
<tr><td>Mobile phase</td><td>Mobile phase A: 0.1% Formic acid: methanol: IPA: GAA (60:35:5:0.5v/v/v/v)
Mobile phase B: Phosphate buffer pH 5
<table>
<tr><th>Time (min)</th><th>Mobile Phase A</th><th>Mobile Phase B</th></tr>
<tr><td>0</td><td>50</td><td>50</td></tr>
<tr><td>5</td><td>70</td><td>30</td></tr>
<tr><td>15</td><td>70</td><td>30</td></tr>
<tr><td>20</td><td>40</td><td>60</td></tr>
<tr><td>25</td><td>70</td><td>30</td></tr>
<tr><td>30</td><td>50</td><td>50</td></tr>
</table></td></tr>
<tr><td>Stationary phase</td><td>Agilent zorbax C18(250×4.6mm,5µm)</td></tr>
<tr><td>Flow rate</td><td>1.0 mL/min</td></tr>
<tr><td>Wavelength</td><td>237 nm, 248 nm</td></tr>
<tr><td>Injection volume</td><td>20µL</td></tr>
<tr><td>Column Temperature</td><td>25°C</td></tr>
</table>

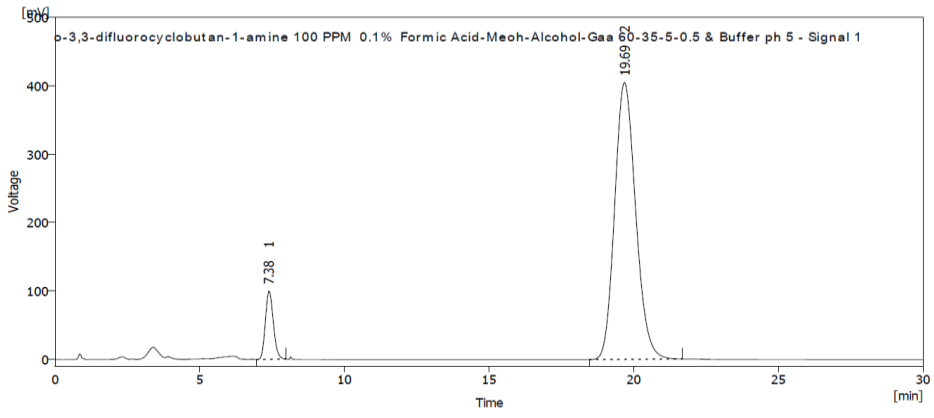

Fig. 3: Chromatogram of Ivosidenib Impurity -1(100 ppm) +Ivosidenib API (400 ppm) in Mobile phase A: 0.1% Formic acid: methanol: IPA: GAA (60:35:5:0.5) Mobile phase B: Phosphate buffer pH 5.

API and impurity both are injected to achieve better separation gradient mode is used. Phosphate buffer was used to maintain stable pH, improve peak symmetry and resolution. Two well separated peaks are observed and baseline separation is achieved.

Method validation

Specificity

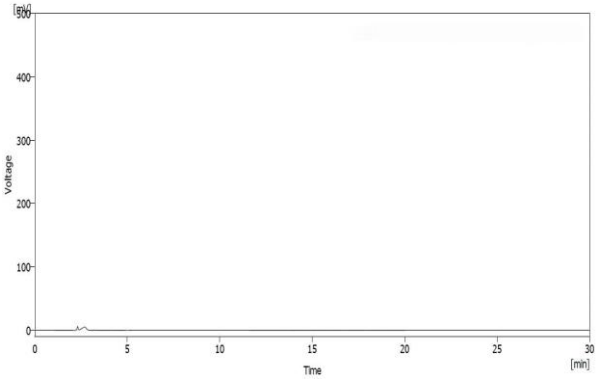

Fig. 4: Blank RP-HPLC chromatogram (diluent).

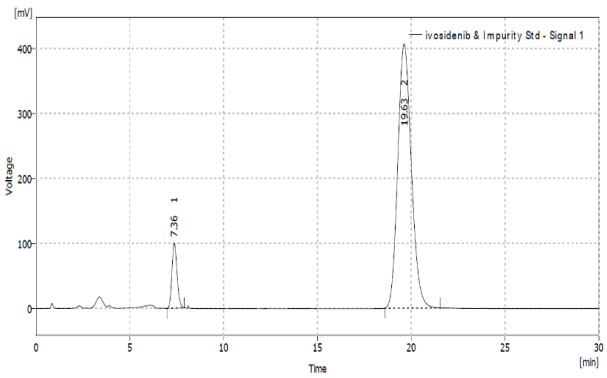

Fig. 5: Chromatogram of standard.

Linearity and Range

Linearity for Ivosidenib API

Table 2: Linearity data for Ivosidenib API.

Sr No.	Concentration(µg/ml)	Area (n=5)
1	200	10225.082
2	300	15375.836
3	400	20437.305
4	500	25589.783
5	600	30710.81
Corelation coefficient	0.999996727	
Y-Intercept	- 6.398	
Slope	51.185	

Linearity for Ivosidenib Impurity-1

Table 3: Linearity data for Ivosidenib Impurity-1.

Sr No.	Concentration(µg/ml)	Area (n=5)
1	50.0	948.554
2	75.0	1431.489
3	100.0	1889.58
4	125.0	2392.977
5	150.0	2853.748
Corelation coefficient	0.999914663	
Y-Intercept	- 5.4808	
Slope	19.088	

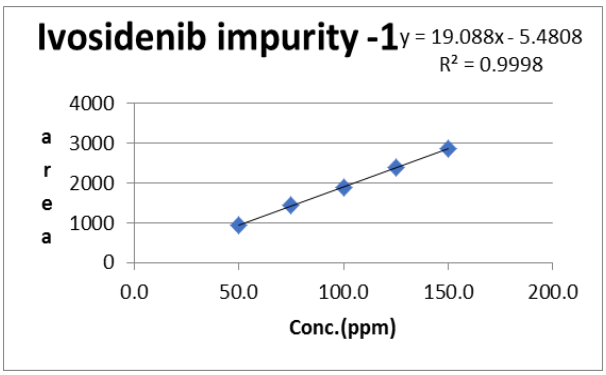

Fig. 6: Calibration curve of ivosidenib Impurity 1.

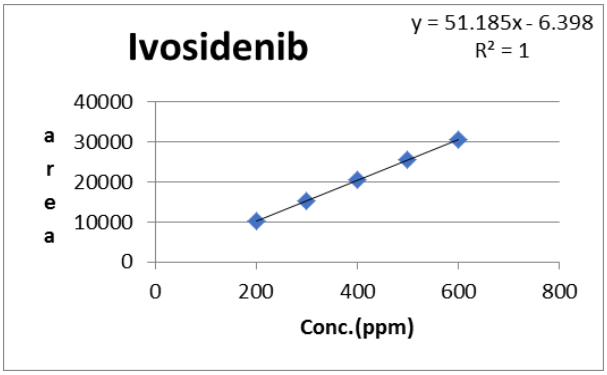

Fig. 7: Calibration curve of ivosidenib API.

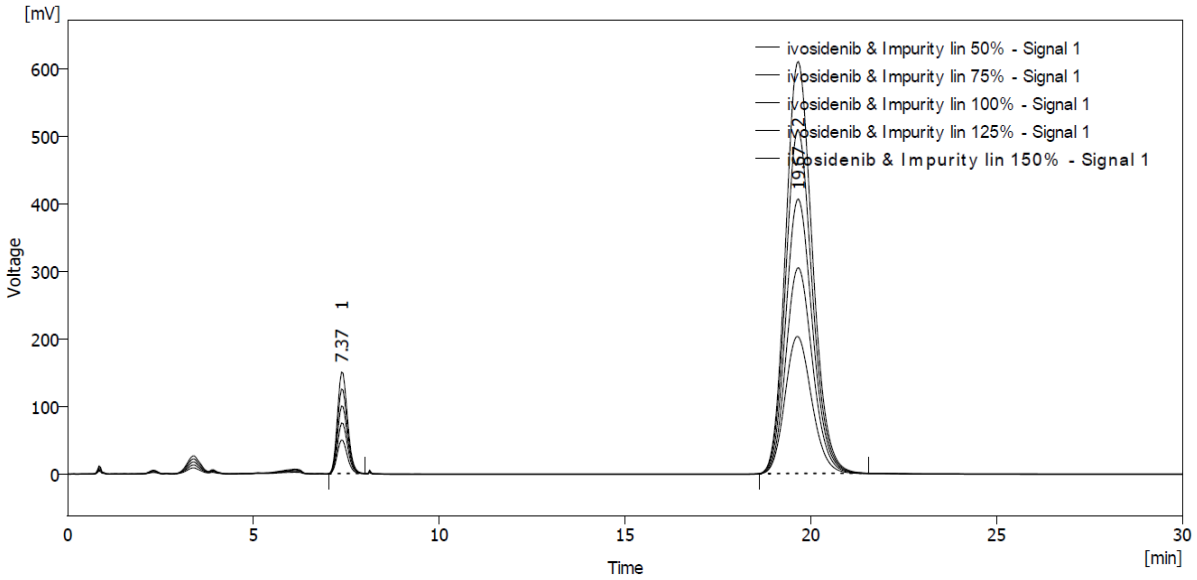

Fig. 8: Overlay Linearity chromatogram of Ivosidenib impurity-1 and Ivosidenib API.

Precision

Repeatability

Table 4: Repeatability data for Ivosidenib Impurity-1, Ivosidenib API.

Ivosidenib impurity -1		Ivosidenib API	
Concentration (µg/ml)	**Area (n=6)**	**Concentration (µg/ml)**	**Area (n=6)**
100(µg/ml)	1890.465	**400(µg/ml)**	20435.639
	1896.548		20436.546
	1875.432		20437.498
	1884.786		20420.458
	1880.927		20468.986
	1892.702		20367.786
Mean	1886.810	**Mean**	20427.819
SD	7.888	**SD**	33.418
%RSD	0.418	**%RSD**	0.164

Intraday Precision

Table 5: Intraday precision for Ivosidenib Impurity-1, Ivosidenib API.

Ivosidenib API			Ivosidenib Impurity 1		
Conc. (µg/ml)	**Area Mean ± SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Area Mean ± SD (n=3)**	**%RSD**
200	10216.484 ± 4.150	0.041	50	946.053 ± 1.911	0.202
400	20444.406 ± 19.797	0.097	100	1879.851 ± 18.560	0.987
600	30708.743 ± 12.518	0.041	150	2853.121 ± 2.575	0.090

Interday Precision

Table 6: Intraday precision for Ivosidenib Impurity-1, Ivosidenib API.

Ivosidenib API			Ivosidenib Impurity 1		
Conc. (µg/ml)	Area Mean ± SD (n=3)	%RSD	Conc. (µg/ml)	Area Mean ± SD (n=3)	%RSD
200	10225.868 ± 5.340	0.052	50	954.613 ± 3.879	0.406
400	20442.994 ± 2.734	0.013	100	1859.597 ± 3.598	0.190
600	30706.323 ± 16.271	0.053	150	2852.607 ± 5.670	0.199

Accuracy (Recovery)

Table 7: Recovery data of Ivosidenib API.

Sr.no.	Level of Spiking	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery	SD	%RSD
1	80%	320	320.48	100.15	100.157	0.198	0.197
		320	319.88	99.96			
		320	321.14	100.36			
2	100%	400	400.91	100.23	100.257	0.027	0.027
		400	401.13	100.28			
		400	401.04	100.26			
3	120%	480	479.41	99.88	99.249	0.092	0.092
		480	479.59	99.92			
		480	480.25	100.05			

Table 8: Recovery data of Ivosidenib impurity -1.

Sr.no.	Level of Spiking	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery	SD	%RSD
1	80%	80	80.63	100.78	101.51	0.65	0.64
		80	81.61	102.02			
		80	81.39	101.74			
2	100%	100	99.87	99.87	99.70	0.57	0.57
		100	100.16	100.16			
		100	99.07	99.07			
3	120%	120	119.45	99.54	100.24	0.77	0.77
		120	120.13	100.11			
		120	121.28	101.07			

Robustness

Table 9: Robustness data of Ivosidenib API.

Sr.no.	Area					
	Flow rate +2 %	Flow rate -2 %	Mobile phase +2%	Mobile phase -2%	pH +0.2	pH -0.2
1	21654.543	20987.159	22312.215	19897.698	21425.369	21730.190
2	21532.649	20879.357	22154.386	19943.297	21298.452	21589.349
3	21420.891	20912.658	22298.462	20041.560	21512.901	21662.561
Mean	21536.028	20926.391	22255.021	19960.852	21412.241	21660.700
SD	116.863	55.198	87.423	73.520	107.826	70.439
%RSD	0.543	0.264	0.393	0.368	0.504	0.325

Table 10: Robustness data of Ivosidenib Impurity -1.

Sr.no.	Area					
	Flow rate +2 %	Flow rate -2 %	Mobile phase +2%	Mobile phase -2%	pH +0.2	pH -0.2

1	1975.262	1823.265	2123.256	1956.397	2018.641	1949.654
2	1963.123	1812.369	2142.565	1934.521	2042.354	1965.234
3	1981.945	1832.786	2160.872	1967.892	2022.894	1955.221
Mean	1973.443	1822.807	2142.231	1952.937	2027.963	1956.703
SD	9.542	10.216	18.810	16.952	12.643	7.895
%RSD	0.484	0.560	0.878	0.868	0.623	0.403

Limit of Detection and Limit of Quantitation

Table 11: LOD data for Ivosidenib Impurity-1, Ivosidenib API.

	Ivosidenib Impurity 1	Ivosidenib API
LOD	1.968	1.54
LOQ	5.963	4.671

Assay

Ivosidenib API

Table 12: Assay data of Ivosidenib API.

Sr no.	Area of sample	% Assay	Average % Assay	SD	%RSD
1	20183.940	98.737	98.584	0.168	0.170
2	20116.059	98.405			
3	20157.872	98.610			

Ivosidenib impurity -1

Table 13: Assay data of Ivosidenib Impurity -1

Sr no.	Area of sample	% Assay	Average % Assay	SD	%RSD
1	0.000(ND)	0.000(ND)	0.000	0.000	0.000
2	0.000(ND)	0.000(ND)			
3	0.000(ND)	0.000(ND)			

CONCLUSION

Based on the experimental results, the proposed method is accurate, novel, simple, precise, linear, sensitive, robust for estimation of ivosidenib impurity 1. The developed method can be effectively used for routine analysis and quality control of ivosidenib and ivosidenib impurity 1.

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