Chiral LC method development for stereo-selective separation and quantification of Ivosidenib and its S,R and R,R isomeric impurities in drug substance and finished product

Tatavarti Bhagya Kumar[1*], Usha Rani N.[2], Tangeti Venkata Swamy[3] and Tulasi Bhavani K.[4]
1. Department of Chemistry, K.B.N. College (Autonomous), Kothapeta, Vijayawada, Andhra Pradesh, 520001, INDIA
2. Department of FED, PVP Siddhartha Institute of Technology, Vijayawada, Andhra Pradesh, 520007, INDIA
3. Department of Chemistry, Tagore Government Arts and Science College (Affiliated to Pondicherry University), Puducherry, 605008, INDIA
4. Department of Engineering Chemistry, Sagi Rama Krishnam Raju Engineering College, Bhimavaram, Andhra Pradesh, 534204, INDIA
*tbhagyakumar@gmail.com

Abstract

A robust and reliable chiral HPLC method was developed and validated for the separation and quantification of ivosidenib and its stereoisomeric impurities (S,R and R,R forms) in pharmaceutical formulations and bulk drug samples. The study optimized various analytical parameters including the mobile phase composition, pH modifiers, flow rate, detector wavelength and column temperature. A mobile phase comprising of methanol and 0.1% formic acid in 65:35 (v/v) ratio, with a flow rate of 0.8 mL/min and a detection wavelength of 248 nm, was identified as optimal. These conditions provided high-resolution symmetrical peaks with retention times of 6.5, 5.7 and 4.8 minutes for ivosidenib, S,R and R,R impurities respectively, ensuring a total analysis time of 10 minutes. System suitability parameters including peak resolution and retention time reproducibility, were within acceptable limits. Linearity studies demonstrated a strong correlation ($R^2 > 0.999$) for ivosidenib (25–250 µg/mL) and its impurities (0.25–2.50 µg/mL). Sensitivity analysis revealed detection limits as low as 0.075 µL/mL for the impurities.

Accuracy studies across multiple concentrations showed recovery rates within 98–102%, with low relative standard deviations, indicating the method's high precision and reproducibility. Robustness testing confirmed the method's reliability. Stability studies validated that ivosidenib and its isomeric impurities remained stable for at least 15 days under both room temperature (25°C) and refrigeration (2°C) conditions. The results demonstrated compliance with pharmacopoeial standards, with ivosidenib content at 99.15% and impurity levels below the detection limit, confirming the stereoselective efficiency of the formulation. The method's reliability, simplicity and applicability to both bulk batch and formulation samples make it an essential tool for pharmaceutical quality assurance.

Keywords: Ivosidenib, isomeric impurities, stereo-selective separation, HPLC quantification.

Introduction

The targeted anticancer drug ivosidenib belongs to isocitrate dehydrogenase-1 (IDH1) inhibitors class designed to treat cholangiocarcinoma (bile duct cancer) and acute myeloid leukemia (myeloid line of blood cells cancer)[9]. It works by inhibiting the mutant IDH1 enzyme, which reduces the accumulation of the oncometabolite 2-hydroxyglutarate (2-HG), a substance that disrupts normal cell differentiation and contributes to cancer progression. The common side effects including nausea, diarrhea, fatigue, leukocytosis and differentiation syndrome are possible during the usage of ivosidenib[4]. In the treatment of cholangiocarcinoma and acute myeloid leukemia, the drug Ivosidenib was awarded as orphan drug by US Food and Drug Administration in the year 2015[10].

Ivosidenib is a chiral molecule that exhibits therapeutic activity primarily in its (S)-enantiomer form. In the process of synthesis of ivosidenib, stereochemical variations can lead to the formation of impurities such as S,R and R,R diastereomers. These impurities arise from changes in the configuration at one or more chiral centers. The S,R impurity retains the same configuration as the active pharmaceutical ingredient (API) at one chiral center but differs at the other. The R,R impurity, on the other hand, has both chiral centers in the opposite configuration compared to the API. These impurities are diastereomers of ivosidenib, differing in their spatial arrangement but sharing the same molecular formula[11].

Quantification of S,R and R,R impurities is critical due to their potential impact on drug safety and efficacy. Diastereomers often exhibit different pharmacological properties, which may lead to reduced therapeutic effectiveness or undesirable toxic effects[2]. Regulatory authorities such as the FDA and EMA mandate that these impurities should be identified, quantified and controlled within specified limits as part of the drug's quality profile. Accurate measurement of these impurities ensures compliance with regulatory guidelines, supports drug safety and validates the manufacturing process[15]. Reversed-phase high-performance liquid chromatography (RP-HPLC) is an

https://doi.org/10.25303/294rjce079085

ideal method for quantifying these diastereomers. RP-HPLC is known for its high specificity and sensitivity, enabling the detection of trace impurities even in the presence of the API and other degradation products. The method is cost-effective, versatile and readily adaptable to different analytical requirements, making it suitable for routine quality control and stability studies[6]. Additionally, RP-HPLC can be optimized with various mobile phases, gradients and detection methods to achieve optimal resolution and accuracy[8]. Developing a robust RP-HPLC method for S,R and R,R impurities is not only a regulatory requirement but also a crucial step in ensuring the safety, efficacy and overall quality of ivosidenib. Such a method provides a reliable and scalable analytical tool for impurity profiling, stability studies and quality assurance in pharmaceutical development and manufacturing[16].

In literature, few methods were reported for quantification of ivosidenib in biological matrixes using HPLC[3,4], LC-MS/MS[3,18] and UPLC-MS/MS[17]. This study planned to propose a simple and stable analytical HPLC method. The structure of ivosidenib, its S,R and R,R isomeric impurities in the study are presented in figure 1.

Material and Methods

Chemicals: The isomeric impurities of ivosidenib and active pharmaceutical ingredient of ivosidenib (98.75 %) along with its 250 mg dosage tablets were procured from Holystics Medicure, Nagpur, Maharashtra. The LR grade chemicals including hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and HPLC grade chemicals including methanol, acetonitrile and water were procured from Merck Chemicals, Mumbai.

Instruments: The HPLC system included a Dionex P580 LPG pump, an ASI-100 T autosampler, an STH 585 column oven and a PDA-100 UV detector. The data was collected and analyzed using a Chromeleon Data System from Dionex Corporation (California, USA). The isomers of ivosidenib were resolved on Chiralpak IG-3 250 mm column (4.6 mm, 3 µm).

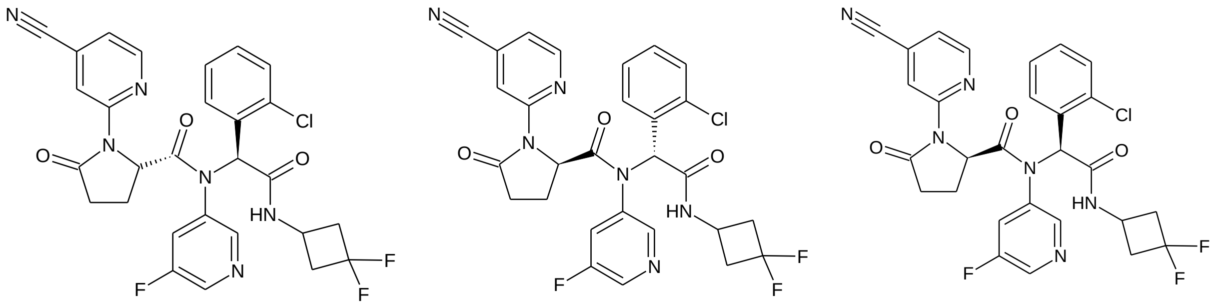
Preparation of standard and system suitability solutions: A solution for system suitability was prepared by dissolving 10 mg of ivosidenib, its S,R and R,R isomeric impurities in a 10 mL volumetric flask containing 5 mL methanol separately. The solution was then passed through a 0.2 µm nylon membrane filter and volume made till mark with methanol. Then the solution was diluted to in the range of 25 to 250 µg/mL separately for standard and 0.25 to 2.50 µg/mL for impurities. The calibration dilutions were prepared by mixing 0.2 mL of standard solution with 0.2 mL of each impurity solution such that the standard solution contains 1 % of each impurity.

Formulation solution: The tablet formulation with 250 mg dosage of ivosidenib was utilized in the process of preparation of tablet formulation solution for HPLC analysis. Initially, tablet weights were accurately weighed and crushed into a fine powder. Then, transfer a specific amount of this powder equivalent to 10 mg of ivosidenib into a 100 mL volumetric flask. 50 mL methanol was added and the mixture was sonicated to dissolve the powder. It was filtered through a membrane filter to remove any un-dissolved particles and volume made till mark. The solution was utilized as formulation analysis solution in the study.

Method development: Before developing the method, the UV iso-absorption wavelength for ivosidenib was identified using a UV spectrophotometer. Various parameters of the chromatographic conditions were adjusted to achieve optimal separation and detection. This included modifying the composition and pH of the mobile phase, altering the flow rate and testing different stationary phases. A standard solution containing ivosidenib, its S,R and R,R isomeric impurities was analyzed under each set of conditions to assess the chromatographic performance[13].

The peak area responses and system suitability parameters including resolution, tailing factor and retention time, were carefully evaluated. Based on these observations, the most suitable conditions were selected for further validation of the method.

Method validation: The HPLC method for ivosidenib, its S,R and R,R isomeric impurities was validated as per ICH guidelines[7].



Ivosidenib S,R isomeric impurity of Ivosidenib R,R isomeric impurity of Ivosidenib

Figure 1: Molecular structure of Ivosidenib, its S,R and R,R isomeric impurities

System suitability was confirmed by injecting an ivosidenib solution with 1% impurities, meeting criteria for tailing factor (<2), resolution (>2), plate count (>3000) and % RSD (<2). Linearity was demonstrated with ten concentrations, achieving an $R^2 \geq 0.999$ for ivosidenib, its S,R and R,R isomeric impurities[14]. Accuracy was validated by % recovery within 50–150% spiked levels whereas precision and ruggedness were evaluated through intra-day and inter-day analyses under varying conditions, showing % RSD <2. Robustness was established by minor changes in temperature, mobile phase pH, flow rate and detector wavelength, with consistent results. The sensitivity was assessed by determining LOD and LOQ concentrations that confirmed the detectability across all studies. Specificity and selectivity were validated through analyses of standards, stress degradation solutions and formulations, ensuring clear resolution without interference.

Stability studies: The stability of ivosidenib, its S,R and R,R impurities sample solutions was evaluated by analyzing three prepared solutions, each containing 100 % recover level concentration under different storage conditions. The first set of solutions was analyzed immediately after preparation to establish baseline readings. Another set was stored at room temperature (25°C) and was analyzed after 15 days, while the third set was kept in a refrigerator at a cooler temperature (2°C) for the same duration. The stability of ivosidenib, its S,R and R,R impurities in these solutions was determined by measuring and comparing the peak areas obtained from the chromatograms under these varying conditions. This approach ensured that any changes in the solutions' stability due to storage conditions were identified and were assessed.

Results and Discussion

Initially, various mobile phases including acetonitrile as organic modifier and acetate, phosphate buffers at different pH levels as pH modifier in the mobile phase were tested. The ammonium acetate and phosphate buffers in the mobile phase proved unsuitable due to unstable baselines caused by poor miscibility. Replacing acetonitrile with methanol and including 0.1% formic acid in mobile phase improved stability. The utilization of triethylamine or diethylamine as pH modifiers to the mobile phase had no significant impact on resolution or peak symmetry and hence basic modifiers were excluded in the mobile phase. After testing different ratios, the optimal mobile phase was determined to be 0.1% formic acid and methanol on 65:35 (v/v).

The flow rate of mobile phase was optimized within the range of 0.6 to 1.2 mL/min. A flow rate of 0.8 mL/min was proved to be ideal based on chromatographic resolution between ivosidenib, its S,R and R,R isomeric impurities. The detector wavelength of 248 nm proved to be ideal for the detection of analytes based on maximum absorption wavelength noticed in UV-visible spectrophotometer. The column temperature was also evaluated between 20°C to 35°C and results proved that the temperature exhibited minimal impact on separation, hence a temperature of 25°C was chosen for the analysis. In these optimized conditions, the method achieved symmetrical peaks and sufficient resolution between the ivosidenib, its S,R and R,R isomeric impurities. The retention time of 6.5 min was noticed for ivosidenib whereas 5.7 min and 4.8 min were noticed for S,R and R,R isomeric impurities respectively with a total analysis time of 10 min (Figure 2).

The specificity of proposed method was evaluated to check for any interference from the sample matrix or excipients. The obtained chromatographic results were verified for its system suitability. The individual analysis of the analytes retention times were correlated with standard and no chromatographic detections were noticed in blank, proving the method specificity. The method passes system suitability results as per guidelines suggesting suitability of method. The system suitability chromatograms were shown in figure 3 and table 1 summarizes the system suitability and specificity results of ivosidenib, its S,R and R,R isomeric impurities in the proposed method.

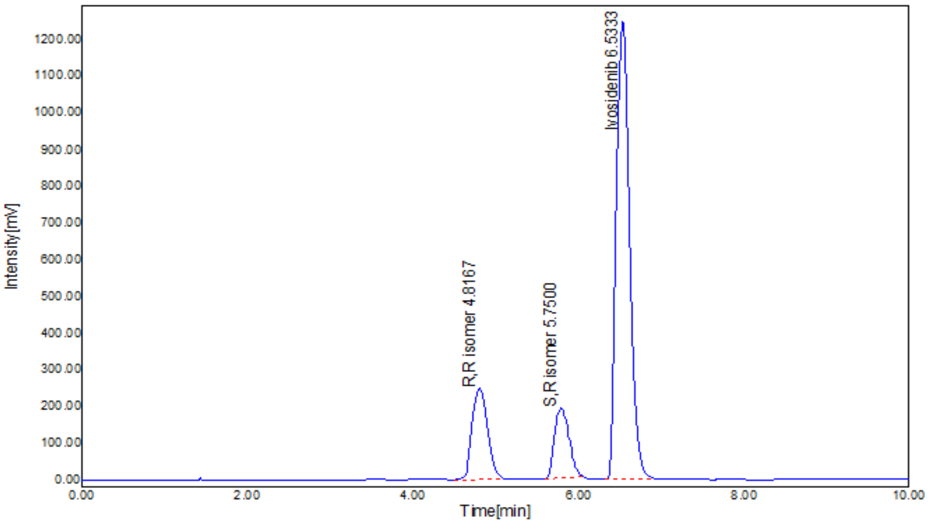
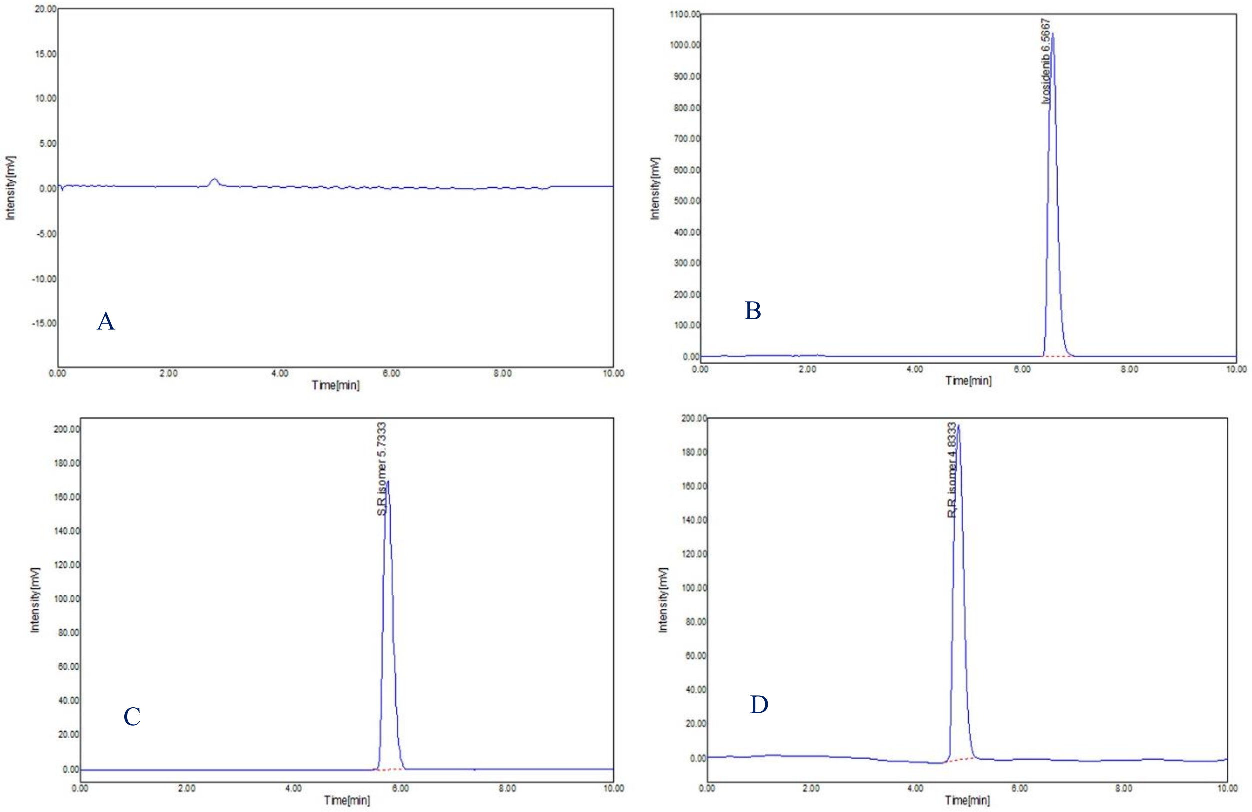


Figure 2: Standard chromatogram of ivosidenib, its S,R and R,R isomeric impurities in the proposed method

Table 1
System suitability results noticed in the proposed method for ivosidenib, its S,R and R,R isomeric impurities

S.N.	**Test**	**Results noticed for**		
		Ivosidenib	**S,R isomer**	**R,R isomer**
1	Retention time (min)	6.5	5.7	4.8
2	Relative retention time	--	0.88	0.73
3	Resolution (Rs)	4.81	4.19	--
4	Theoretical plates (TP)	11326	8701	5297
5	Tail factor (TF)	1.03	1.01	0.97



A) blank chromatogram; B) ivosidenib standard; C) S,R isomeric impurity chromatogram; D) R,R isomeric impurity
Figure 3: System suitability chromatograms noticed in the proposed method for ivosidenib, its S,R and R,R isomeric impurities

Table 2
Linearity and Sensitivity results noticed in the proposed method for ivosidenib, its S,R and R,R isomeric impurities

S.N.	**Test**	**Results noticed for**		
		Ivosidenib	**S,R isomer**	**R,R isomer**
1	Linearity (µL/mL)	25 to 250	0.25 to 2.50	0.25 to 2.50
2	correlation coefficient (r)	0.9995	0.9991	0.9993
3	Intercept/ (mAU)	4438.9	2644.5	555.82
4	Slope/ (mAU µL/mL)	7795	47487	39080
5	LOQ (µL/mL)	25	0.25	0.25
6	LOD (µL/mL)	7.5	0.075	0.075

The linearity of the proposed method was tested across ten concentration levels, ranging from 25 to 250 µg/mL for ivosidenib and 0.25 to 2.50 µg/mL for impurities. The linear range covers approximately 10-100% of the analyte's nominal range for ivosidenib. Calibration curves were created using linear least squares regression. The average regression equations obtained (n = 3) along with sensitivity results were tabulated in table 2. The linear correlation coefficients exceeding 0.999 for both ivosidenib and impurities suggest the accurate fit of the method. The sensitivity results suggest that the method can detect isomeric impurities up to a very minimal concentration of 0.075 µL/mL suggesting the high sensitivity of method.

The accuracy of the method for determining ivosidenib, its S,R and R,R isomeric impurities was assessed at three concentration levels i.e. 50%, 100% and 150% to the target concentration of 50 μL/mL for ivosidenib and 0.5 μL/mL for impurities. The % recovery in each injection results and % RSD of each spiked level were assessed for ivosidenib, its S,R and R,R impurities. The results as detailed in table 3 demonstrate that the % recovery for all measurements was found within 98-102%, indicating that the method provides consistent and reproducible results under the tested conditions. Additionally, the method's accuracy was confirmed by comparing the theoretical concentrations with the experimentally obtained values, which showed minimal differences. These results indicate the method's high accuracy and consistency across the tested concentration range.

In robustness study, the statistical analysis revealed no significant difference between the results obtained under the optimized analytical conditions of the method and those from experiments where certain parameters were varied. The minor changes in analytical parameters did not have a noticeable effect on peak separation, shape and area response. These changes did not exhibit any impact on peak integration process which in turn influenced the quantification of the isomers. This finding invalidates the method and highlights that the nominal variation in proposed conditions such as detection wavelength, solvent composition and flow rate also ensure accurate peak separation, integration and reliable quantification. Table 4 summarizes the results noticed in robustness study of ivosidenib, its S,R and R,R isomeric impurities in the proposed method.

Table 3
Accuracy results noticed in the proposed method for ivosidenib, its S,R and R,R isomeric impurities

S.N.	Component	Accuracy level	Concentration prepared (μL/mL)	Concentration recovered (μL/mL)	% Recovery	% RSD of recovery
1	Ivosidenib	50 %	75	73.69 ± 0.251	98.25	0.26
2		100 %	100	98.63 ± 0.586	98.63	0.59
3		150 %	125	124.68 ± 0.334	99.74	0.33
4	S,R impurity	50 %	0.75	0.738 ± 0.517	98.39	0.53
5		100 %	0.100	0.099 ± 0.881	99.15	0.89
6		150 %	1.25	1.238 ± 0.361	99.01	0.36
7	R,R impurity	50 %	0.75	0.740 ± 0.291	98.67	0.29
8		100 %	0.100	0.099 ± 0.224	99.04	0.23
9		150 %	1.25	1.254 ± 0.693	100.34	0.69

n = 3

Table 4
Robustness results noticed in the proposed method for ivosidenib, its S,R and R,R isomeric impurities

S.N.	Robustness parameter	Component	Results		
			% change	TP	Rs
1	Mobile phase composition as formic acid and methanol on 65:35 (v/v)	Ivosidenib	0.85	11838	4.83
2		S,R impurity	0.23	8628	4.13
3		R,R impurity	0.91	5359	--
4	Mobile phase composition as formic acid and methanol on 65:35 (v/v)	Ivosidenib	0.57	11758	4.89
5		S,R impurity	0.66	8666	4.15
6		R,R impurity	0.49	5476	--
7	Mobile phase flow rate of 0.75 mL/min	Ivosidenib	0.52	11820	4.88
8		S,R impurity	0.47	8637	4.09
9		R,R impurity	0.36	5318	--
10	Mobile phase flow rate of 0.85 mL/min	Ivosidenib	1.05	11822	4.80
11		S,R impurity	0.99	8798	4.09
12		R,R impurity	0.82	5456	--
13	Detector wavelength at 243 nm	Ivosidenib	1.11	11803	4.89
14		S,R impurity	0.47	8876	4.15
15		R,R impurity	1.03	5530	--
16	Detector wavelength at 253 nm	Ivosidenib	0.25	12189	4.79
17		S,R impurity	0.33	8858	4.06
18		R,R impurity	0.73	5454	--

n = 3

The stability of ivosidenib, its S,R and R,R impurities solutions was assessed by analyzing them immediately after preparation, after 15 days of storage at room temperature (25°C) and after 15 days of refrigeration at a cooler temperature (2°C). The results showed that there was no degradation of the drug under any of these conditions. The peak areas for ivosidenib, its S,R and R,R impurities obtained from the chromatographic analysis were consistent across all three storage conditions. Statistical analysis confirmed that there were no significant differences between the peak areas ($p > 0.05$), demonstrating that analytes remain stable for at least 15 days when stored at room temperature or under refrigeration. This indicates that the drug's chemical integrity is maintained over this period.

The developed chiral method was utilized for assay of ivosidenib in tablets to assess its effectiveness for real time applications in routine analysis. Three batches of fixed-dose combination tablets containing 250 mg of ivosidenib were analyzed. The analysis revealed that the content of isomeric impurities in the tablets ranged from less than the detection limit while standard ivosidenib was found to be 99.15%. These findings indicated that the impurities levels in each sample were less than detection limit, confirming the sterio selective efficiency of ivosidenib and its isomeric forms. This study marks the first instance of a chiral method being successfully developed and applied to separate and quantify ivosidenib, its S,R and R,R impurities in tablet formulations. The method is suitable for routine quality control and offers the capability to assess the quantity of ivosidenib.

The study findings correlated with literature. Our method is essential for ensuring the safety, efficacy and quality of ivosidenib products, as the presence of stereoisomeric impurities can influence the pharmacological activity and toxicity profile of the drug.

Conclusion

This study aimed to develop a reliable and robust chiral analytical method for the separation and quantification of ivosidenib and its stereoisomeric impurities such as S,R and R,R forms, in pharmaceutical formulations and bulk drug samples. Various mobile phases and pH modifiers were tested, with the optimal conditions identified as a mixture of methanol and 0.1% formic acid in 65:35 (v/v) ratio, a flow rate of 0.8 mL/min, a detector wavelength of 248 nm and a column temperature of 25°C. Under these optimized conditions, the method achieved excellent chromatographic resolution and symmetrical peaks for ivosidenib and its isomeric impurities, with retention times of 6.5, 5.7 and 4.8 minutes for ivosidenib, S,R and R,R impurities respectively, within a total analysis time of 10 minutes.

The method demonstrated high specificity, as no interference from excipients or the sample matrix was observed. System suitability tests and linearity studies confirmed the method's reliability, with correlation coefficients exceeding 0.999 for all analytes and sensitivity down to 0.075 µL/mL for impurities. The developed method bridges a critical gap in quality control and regulatory evaluation, ensuring the safety, efficacy and quality of ivosidenib formulations. By enabling accurate quantification of stereoisomeric impurities, the method supports therapeutic consistency and compliance with pharmaceutical standards, while its robustness and simplicity make it suitable for routine application in quality control laboratories.

References

1. Chen M., Xu J., Chen F., Zhou Q., Wang S. and Han A., Validated UPLC-MS/MS method for the determination of ivosidenib in rat plasma: Application to a pharmacokinetic study, *Acta Chromatogr*., **35**, 227 **(2023)**

2. Dhangar K.R., Jagtap R.B., Surana S.J. and Shirkhedkar A.A., Impurity profiling of drugs towards safety and efficacy: theory and practice, *J Chil Chem Soc.*, **62**, 3543 **(2017)**

3. Dittakavi S., Jat R.K., Mallurwar S.R., Jairam R.K. and Mullangi R., Validated LC-ESI-MS/MS method for the determination of ivosidenib in 10 µL mice plasma: application to a pharmacokinetic stud, *ADMET DMPK*, **7**, 131 **(2019)**

4. Fan B., Dai D., DiNardo C.D., Stein E., Stéphane de B., Eyal C. A., Hua L., Guowen L., Ian Lemieux, Samuel V.A. and Hua Y., Clinical pharmacokinetics and pharmacodynamics of ivosidenib in patients with advanced hematologic malignancies with an IDH1 mutation, *Cancer Chemother Pharmacol*, **85**, 959 **(2020)**

5. Gando Y. and Yasu T., A Simple HPLC-UV Method for Ivosidenib Determination in Human Plasma, *J Chromatogr Sci*, **62**, 580 **(2024)**

6. Girija K.S., Kasimala B.B. and Anna V.R., a new high-performance liquid chromatography method for the separation and simultaneous quantification of eptifibatide and its impurities in pharmaceutical injection formulation, *Int. J. Appl. Pharm*, **13**, 165 **(2021)**

7. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Impurities in New Drug Substances, Q3A(R2), Version 4, 1 **(2006)**

8. Mallu U.R., Anna V.R. and Kasimala B.B., rapid stability indicating HPLC method for the analysis of leflunomide and its related impurities in bulk drug and formulations, *Turk J Pharm Sci*, **16**, 457 **(2019)**

9. Popovici-Muller J. et al, Discovery of AG-120 (Ivosidenib): A First-in-Class Mutant IDH1 Inhibitor for the Treatment of IDH1 Mutant Cancers, *ACS Med Chem Lett*, **9**, 300 **(2018)**

10. Public summary of opinion on orphan designation, Ivosidenib for the treatment of acute myeloid leukaemia, EU/3/16/1802, European Medicines Agency (EMA), 1 **(2017)**

11. Somana Siva Prasad, Kasimala B.B. and Anna V.R., QBD-based analytical method development and validation for the separation and quantification of agomelatine and its impurities in solid oral dosage forms using HPLC, *Rasayan J Chem*, **14**, 2183 **(2021)**

12. Tummala Sambasiva Rao and Amgoth Krishnamanjari Pawar, development of GC-MS/MS method for simultaneous estimation of four nitrosoamine genotoxic impurities in valsartan, *Turk J Pharm Sci.*, **19**, 455 **(2022)**

13. Tummala Sambasiva Rao and Amgoth Krishnamanjari Pawar, LC-MS/MS approach for the quantification of five genotoxic nitrosoimpurities in varenicline, *J. Pharm. Res*., **26**, 1685 **(2022)**

14. Tummala Sambasiva Rao, Gorrepati Naveena and Tatapudi Hemant Kumar, Head Space GC-MS/MS method for quantification of five nitrosoamine- genotoxic impurities in metformin HCl, *Curr. Pharm. Anal*., **20**, 944 **(2024)**

15. Vibhuti Raj Kamal, Kumar Yatin and Pramanik Avijit, *In silico* analysis of putative hemolysin proteins in the genome of Vibrio alginolyticus ATCC 17749 and their structure prediction, *Res. J. Biotech.*, **19(1)**, 55-58 **(2024)**

16. Vineetha Rosireddy and Manikandan Krishnan, A sustainable RP-HPLC method for simultaneously estimating aceclofenac, paracetamol and serratiopeptidase with AQbD optimization, *Green Analytical Chemistry*, **11**, 100171 **(2024)**

17. Yin F., Yu S., Narayanaswamy R., Mangus H., McCourt E. and Liu G., Quantitation of ivosidenib in human plasma via LC-MS/MS and its application in clinical trials, *Bioanalysis*, **13**, 875 **(2021)**

18. Zakkula A. et al, Validated HPLC method for simultaneous quantification of mutant IDH1/2 inhibitors (enasidenib, ivosidenib and vorasidenib) in mouse plasma: Application to a pharmacokinetic study, *Biomed Chromatogr.*, **33**, 4658 **(2019)**.

(Received 14th December 2024, accepted 16th January 2025)