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A New Validated Stability Indicating RP-HPLC Method for Estimation of Vorasidenib in Bulk and in its Pharmaceutical Dosage Form

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Abstract

For the estimation of Vorasidenib in bulk and in its pharmaceutical dosage form, a reversed phase high-performance liquid chromatographic approach has been designed and validated in the current work. The separation of Vorasidenib was achieved on Waters Alliance-e2695, by using an X-Bridge Phenyl column (250x4.6mm, 5µ) by eluting with a mobile phase consisting of a mixture of acetonitrile and 0.1% Trifluoroacetic acid in the ratio of 40:60v/vat a flow rate of 1.0 mL/min; detection was carried out by absorption at 234 nm using a photodiode array detector at ambient temperature. The total run time set for the elution of the compound was 5 min. Under the optimised chromatographic conditions, the retention time was obtained at 2.855 min. The current analytical technique validation was conducted in accordance with ICH standards (ICH, Q2R1). The concentration range forVorasidenib in the linearity study was found to be 20–120 µg/mL and the coefficient of variance was found to be 0.9999. The percentage recovery was found to be 99.6-100.3%. LOD and LOQ were found to be 0.48 µg/mL and 1.6 µg/mL respectively. The developed method was also applied to monitor the forced degradation studies on the drug for testing for its ability to resolve the drug from their degradation products. The specificity of the developed method was evaluated by applying acid, base, oxidation, thermal, photolytic and neutral stress conditions to the drug. It was concluded that the estimation of Vorasidenib in bulk and its pharmaceutical dosage form was found to be successfully conducted by using the method.

Keywords: RP-HPLC, PDA Detector, Vorasidenib, Method Validation

Introduction

Vorasidenib (Figure 1) is a targeted cancer drug that treats astrocytoma or oligodendroglioma types of brain tumours in adults and children. The drug inhibits the enzymes of Isocitrate dehydrogenase-1(IDH1) & Isocitrate dehydrogenase-2 (IDH2). It is available in 20 & 40mg of oral tablet formulation. The available brand is Voranigo. Chemically it is 6-(6-Chloro pyridine-2-yl]-N2, N4-bis[(2R)-1,1,1-trifluoro propan-2yl]-1,3,5-triazine-2,4-diamine. It's an empirical formula $C_{14}H_{13}ClF_6N_{6s}$ and molecular weight of 414.74g/mol (1-10).

A literature survey revealed that only one analytical method has been reported for estimating Vorasidenib in bulk and its pharmaceutical dosage form. The reported method is RP-HPLC (11). The present study aimed to develop a simple, sensitive, rapid, and precise RP-HPLC method for estimating Vorasidenib. The analytical method was validated according to ICH validation parameters.

Materials and Methods

Chemicals and reagents

A pure sample of Vorasidenib was obtained from Servier India Private Limited, Mumbai. The marketed formulation of

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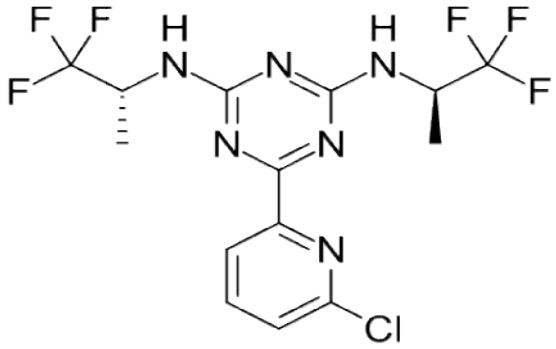

Figure 1: Chemical structure of Vorasidenib

Vorasidenib (Voranigo 40 mg/tablet) was purchased from local pharmacy store. Acetonitrile (HPLC grade) was procured from Rankem®, India, Trifluoroacetic acid (HPLC grade) was procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India, and HPLC graded water and methanol were purchased from Merck Specialties Pvt. Ltd, Mumbai, India. The study made use of water that has been purified using a Milli-Q system.

Instrumentation

The analysis was performed by using a chromatographic system, Waters alliance HPLC system, Quaternary gradient pump of Waters Alliance-e2695 series equipped with an auto sampler injector with 10µL is injector loop by using a photo diode array detector and running on E-Z Chrome software with a reverse phase by using Phenyl column 250 x 4.6 mm internal diameter, 5µm particle size. UV-Visible Spectrophotometer (LAB INDIA-3200+), Shimadzu electronic balance (AX-200) was used for weighing purpose. Ultrasonicator (Citizen) and Class "A" volumetric glassware were employed for the study.

Chromatographic conditions

Vorasidenib was analyzed with Phenyl column (250x4.6mm, 5µ Particle size) for the chromatographic separation and column was maintained at ambient temperature. The mobile phase was composed of a mixture of Acetonitrile and0.1% Trifluoro acetic acid in the ratio of 40:60% v/vand it was delivered at a flow rate of 1.0 mL/min and detection was monitored at 234 nm with PDA detector. Mobile phase was used as diluent. Injection volume was 10 µL. The run time was 5 min. The retention time of Vorasidenib was found to be 2.855 min.

Preparation of TFA buffer solution

One millilitre of tri fluoro acetic acid is dissolved in one litre of HPLC graded water and filtered through 0.45µ nylon syringe filter.

Preparation of mobile phase

Mobile phase was prepared by mixing 0.1% tri fluoro acetic acid and acetonitrile taken in the ratio 60:40. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Diluent

Acetonitrile is used as a diluent.

Preparation of standard solution

Accurately weighed and transferred 8mg of Vorasidenib working standard into a 10 mL clean and dry volumetric flask and diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent to get Stock solution. Further pipetted 1mL of the above stock solutions into a 10 mL volumetric flask and diluted up to the mark with diluent to get 80ppm of Vorasidenib.

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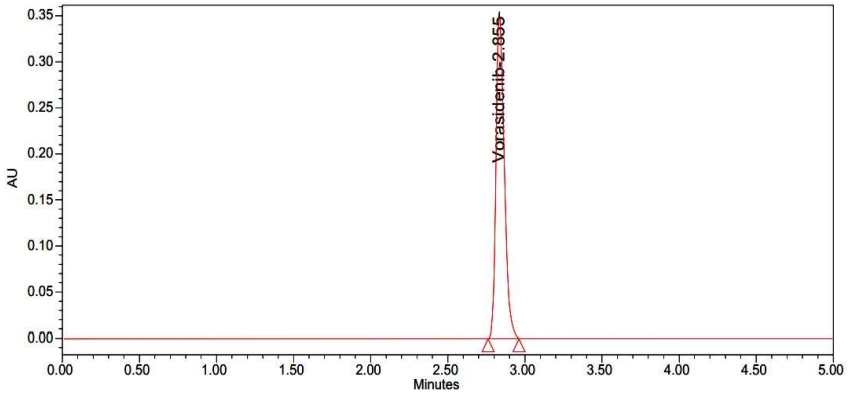

Figure 2: Chromatogram of Vorasidenib standard

Sample Solution Preparation

Accurately weighed and transferred an equivalent to 18.8mg of Vorasidenib sample into a 10mL clean dry volumetric flask diluent was added and sonicated for 30 min to dissolve it completely and made volume up to the mark with the same solvent. Then it is filtered through 0.45-microninjection filter. Further pipetted 1 mL of the above stock solutions into a 10mL volumetric flask and diluted up to the mark with diluent to get 80ppm of Vorasidenib.

Method Development and Optimization

Several mobile phases of various compositions were examined to provide an optimization of chromatographic conditions for system suitability such as retention time, tailing factor, peak resolution, and theoretical plate count etc. Initially, a mixture of formic acid and acetonitrile in ratios of 20:80, 30:70, and 40:60 was explored. Additionally, mixtures of 0.1% Trifluoroacetic acid (TFA) and Acetonitrile in the ratios of 50:50 and 40:60 were also tested. The optimization process included adjusting the flow rates and mobile phase ratios to achieve the best separation. Ultimately, the mobile phase consisting of 0.1% TFA and acetonitrile (60:40, v/v) at a flow rate of 1.0 mL/min was found to provide the most satisfactory chromatographic performance. This system resulted in well-resolved peaks with good shape and was selected as the optimal method for the determination of Vorasidenib both in bulk and in its pharmaceutical dosage form.

Method Validation

The method was validated for Specificity, linearity, accuracy, precision, limit of detection, limit of quantification and robustness by following procedures as per ICH guidelines (12).

Results and Discussion

The developed method was optimised for the determination of Vorasidenib in bulk and in its pharmaceutical dosage form. Resulted in peak with good shape and well resolved. The results of the optimized HPLC conditions were shown in Figure 2 and Table 1.

The proposed method was validated according to the ICH Q2R1 guidelines which include system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

System suitability

In the present study, parameters such as plate count (N), tailing factor (T), resolution (Rs) and reproducibility (%RSD) are determined from replicate injection of standard solution. The acceptable limit of %RSD is less than 2%. Table 2 shows that system suitability results obtained in the present study for Vorasidenib.

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Table 1: Optimized chromatographic conditions

Parameter	Observation
Instrument used	Waters Alliance e-2695 HPLC
Column	Waters X-Bridge Phenyl (250x4.6 mm, 5 µm)
Mobile Phase	Acetonitrile and 0.1% Tri-fluoro acetic acid (40:60)
Flow Rate	1 mL/min
Runtime	5 min
Injection volume	10 µL
Detection Wavelength	234 nm
Temperature	Ambient (25°C)
Mode of separation	Isocratic mode

Table 2: System suitability parameters for Vorasidenib

S. No.	Parameter	Vorasidenib
1	Retention time (min)	2.855
2	Plate count	11958
3	Tailing factor	0.90
4	Resolution	----
5	%RSD	0.21

Specificity

The specificity of an analytical method is to determine the effect of excipients and other additives that are generally present in the formulation. In this study, the method was evaluated by injecting 10 µL of placebo, standard solution and sample solution into the HPLC system. The test results obtained were contrasted with the results of the standard drug. The chromatograms were checked for the interference peaks shown in Figure 3. Retention time of Vorasidenib wasfound 2.855 min. We did not find any interfering peaks in blank and placebo at retention times of these drug in this method. So, this method was said to be specific.

Linearity and Range

The linearity of the method was determined at six concentration levels ranging from 50-120 µg/mL for the drug. Evaluation of the drug was performed with a PDA detector at 234 nm, peak area was recorded for all the peaks. The linearity of the method was evaluated by linear regression analysis. The results shown that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. Linear regression data and linearity curves for both analytes were given in Table 3 and Figure 4.

Table 3: Results of Linearity for Vorasidenib

S. No.	Vorasidenib	
	Concentration (µg/mL)	Peak area
1	20.00	842684
2	40.00	1569102
3	60.00	2334947
4	80.00	3158539
5	100.00	3934065
6	120.00	4784103
Regression equation	y = 39558.05 x+4294.14	
Slope	39558.05	
Intercept	4294.14	
R^2	0.9999	

Accuracy

The accuracy of the method was evaluated by standard addition method. Known amounts of the reference standard solution were added at three different concentration levels to the working standard solution of the drug. The solutions were analysed (n=3) and the mean recovery of the drug at each concentration level was computed and the %RSD was calculated. The studies were performed for Vorasidenib at three different concentration levels (50, 100 and 150%). 10 µL of the samples were injected into the HPLC and the percent recovery and percent RSD were calculated. The corresponding results obtained in the experiments were shown in Table 4.

Precision

In this study, the precision of the method is determined as Intra-day precision and Inter-day precision. The former is determined by injecting 10 µL of the sample for

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six times and noting the peak areas of the analytes, taking the average value and finding out the percent RSD. Similarly, the inter-day precision is determined by injecting the sample six times each on two consecutive days, taking the overall average value and calculating the %RSD. The results of the above parameters obtained are summarized in Table 5.

Robustness

It is the capacity of a method to remain unaffected by small deliberate variations in method parameter values. The relevant values obtained in the present study by changing the flow rate, mobile phase composition is presented in Figure 5 and Table 6.

Limit of Detection and Limit of Quantification:

The LOD and LOQ values of Vorasidenib from standard deviation of the response and the slope values obtained from their linearity curve, LOD and LOQ for

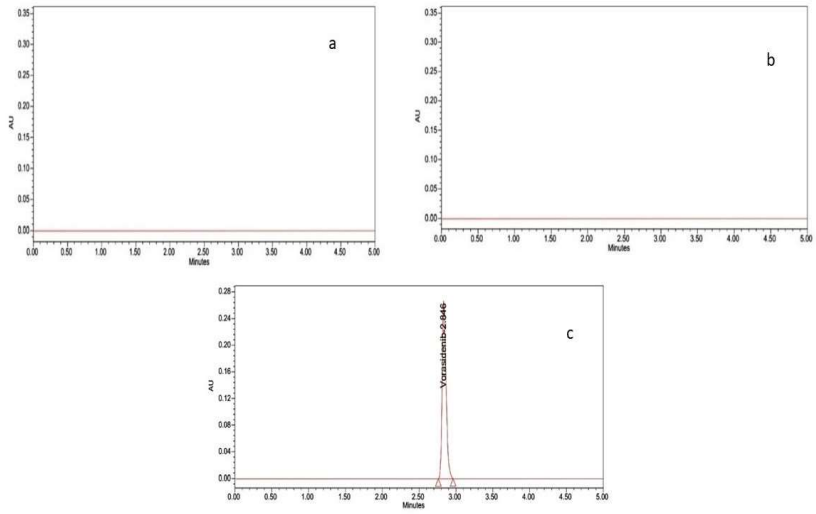

Figure 3: (A) Chromatogram of Blank; (B) Chromatogram of Placebo; (C) Optimized chromatogram

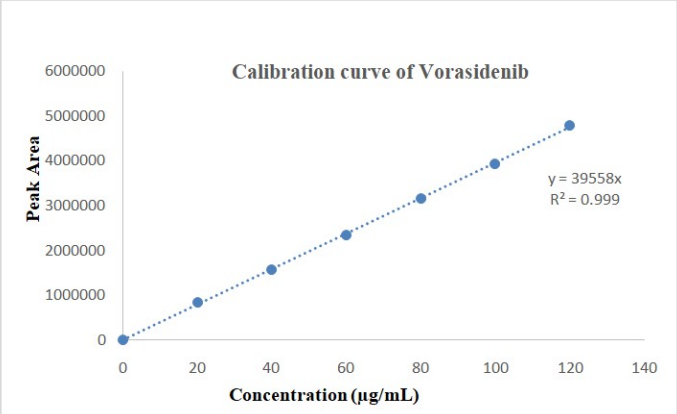

Figure 4: Calibration curve for Vorasidenib at 234 nm

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Table 4: Accuracy results of Vorasidenib

Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean %Recovery
50%	1586487	4.0	4.02	100.5	100.3
	1574096	4.0	3.98	99.5	
	1593432	4.0	4.03	100.8	
100%	3165234	8.0	8.01	100.1	99.6
	3130265	8.0	7.92	99.0	
	3148796	8.0	7.97	99.6	
150%	4722130	12.0	11.95	99.6	99.7
	4698521	12.0	11.89	99.1	
	4758103	12.0	12.04	100.3	

Table 5: Precision study results of Vorasidenib

S. No.	Concentration Vorasidenib (µg/mL)	System Precision	Method Precision
		Peak area	Peak area
1	80	3161273	3145206
2	80	3149174	3178521
3	80	3165906	3180740
4	80	3155689	3162963
5	80	3163622	3140687
6	80	3166453	3158842
Mean		3160353	3161160
S. D		6727.58	16535.287
%RSD		0.213	0.52

Vorasidenib was shown in Figure. 6 and Table 7, Which shows the sensitivity of the method.

Estimation of drug in the tablet dosage form:

The proposed validated method was applied for the determination of Vorasidenib in commercial formulations. The % assay was found to be 99.5 %, Table 8 depicts the assay's results.

Forced degradation studies on the drugs:

In the present study, forced degradation studies were carried out by subjecting the mixed standard solutions of Vorasidenib to acidic (1N HCl), alkali (1N NaOH), oxidative (3% H_2O_2), dryheat (60^0C/ 6h), photolytic (UV chamber/7 Days) and Neutral degradation (HPLC grade water for 6 h at 60^0C) conditions in a laboratory setting to evaluate stability, and the results and data are compared with standard chromatograms. The results obtained from the above experiments indicate that certain amount of degradation of the drug was observed in case of acid and alkaline, peroxide and thermal stress conditions, whereas small extra peaks were found in the relevant chromatograms. It also observed that the purity angle was always less than the purity threshold and it indicates the proposed method was homogenous and can identify the degraded peaks. The chromatograms and drug degradations data obtained in the study were shown in Figure 7 and Table 9.

Estimation of Vorasidenib

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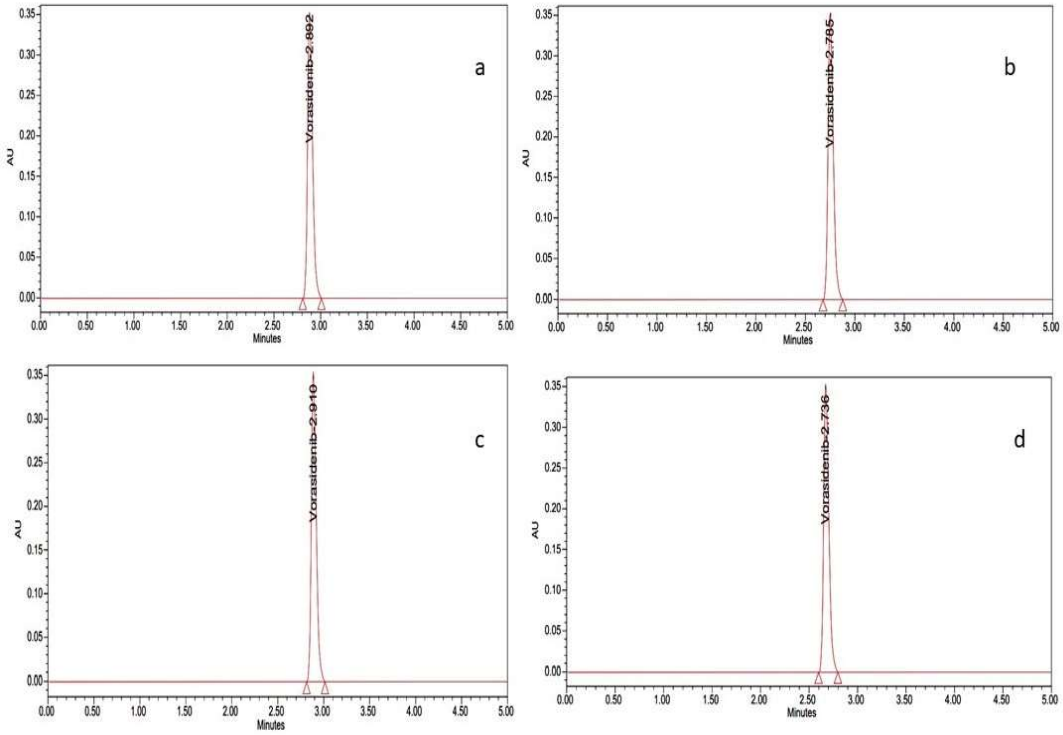

Figure 5: (A) Chromatogram for less flow rate (0.9mL); (B) Chromatogram for more flow (1.1 mL); (C) Chromatogram for less Organic Phase (36:64); (D) Chromatogram for more Organic Phase (44:56)

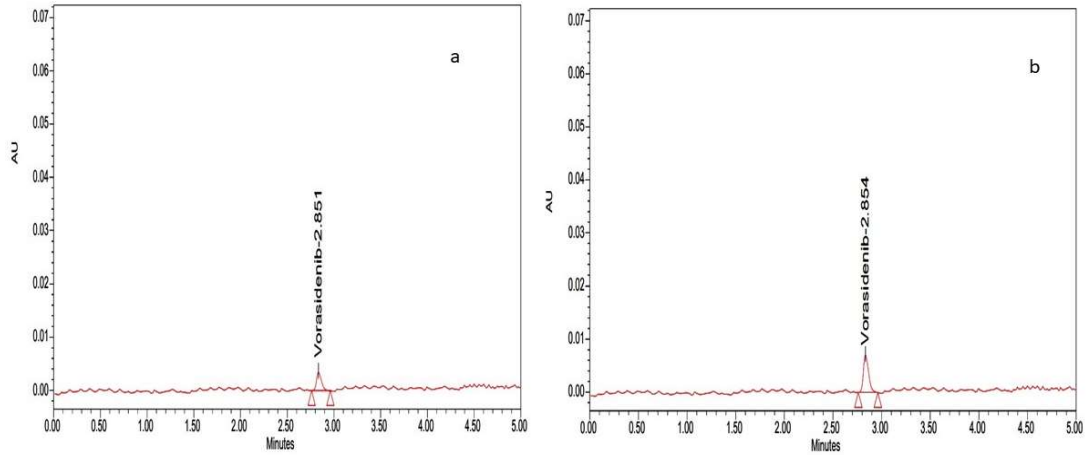

Figure 6: (A) Chromatogram for LOD; (B) chromatogram for LOQ

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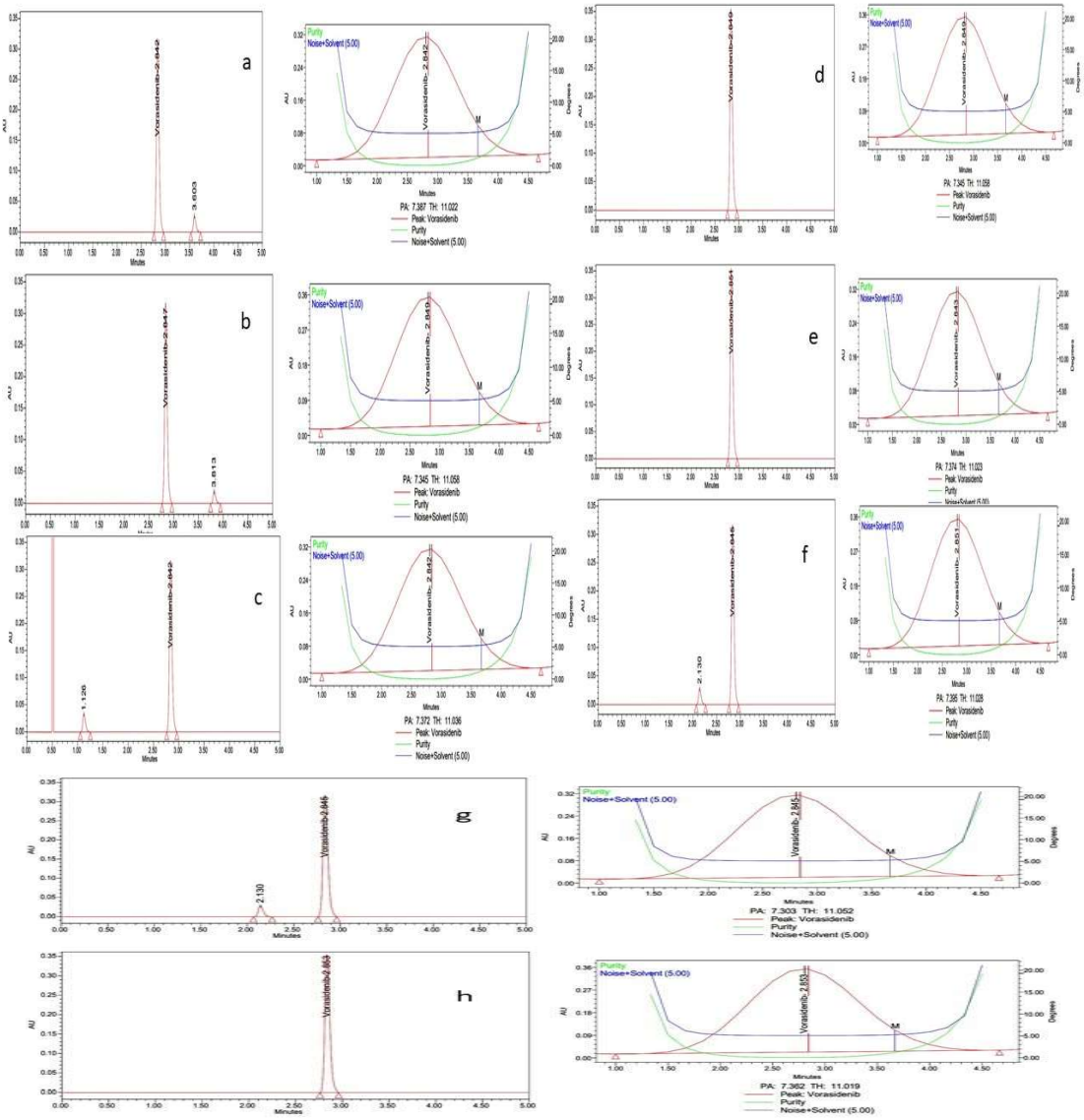

Figure 7: Forced Degradation study chromatograms of Vorasidenib. (A) Chromatogram of acid degradation and Purity Plot of Acid degradation; (B) Chromatogram of alkali degradation and Purity Plot of Alkali degradation; (C) Chromatogram of peroxide degradation and Purity plot of peroxide degradation; (D) Chromatogram of reduction degradation and Purity plot of reduction degradation; (E) Chromatogram of hydrolysis degradation and Purity plot of hydrolysis degradation; (F) Chromatogram of control degradation and Purity plot of control degradation; (G) Chromatogram of thermal degradation and Purity Plot of thermal degradation; (H) Chromatogram of photolytic degradation and Purity Plot of photolytic degradation

Estimation of Vorasidenib

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Table 6: Robustness results of Vorasidenib by HPLC

Parameter	Condition	Retention time (min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow (0.9 mL)	2.892	3111575	0.98	12053
	Actual flow (1 mL)	2.855	3161273	0.90	11958
	More flow (1.1 mL)	2.785	3170798	0.86	11871
Organic Phase change	Less Org. (36:64)	2.910	3127258	0.95	12097
	Actual (40:60)	2.847	3149174	0.92	11962
	More Org. (44:56)	2.736	3196341	0.83	11824

Table 7: Sensitivity parameters (LOD & LOQ) by HPLC

Name of drug	LOD (µg/mL)	S/N	LOQ (µg/mL)	S/N
Vorasidenib	0.48	3	1.6	10

Table 8: Assay results of Vorasidenib

Brand	Drug	Area	Average sample area	Std. Wt. (mg)	Sample wt. (mg)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
Voranigo	Vorasidenib	3139682 3146584	3143133	8	18.8	40	99.98	7.96	99.5

Table 9: Forced Degradation results for Vorasidenib

Condition	Sample Weight In mg	Area Counts Injections	Mean Area Count	% Label Claim	Purity Angle	Purity Threshold	% Degra-dation	Pass/Fail
Control	18.8	3159154	3159154	100	7.395	11.028	0	Pass
Acid	18.8	2775423	2775423	87.9	7.387	11.022	12.1	Pass
Alkali	18.8	2820913	2820913	89.3	7.380	11.035	10.7	Pass
Peroxide	18.8	2705036	2705036	85.6	7.372	11.036	14.4	Pass
Reduction	18.8	3096834	3096834	98.0	7.345	11.058	2.0	Pass
Thermal	18.8	2758611	2758611	87.3	7.303	11.052	12.7	Pass
Photolytic	18.8	3035306	3035306	96.1	7.362	11.019	3.9	Pass
Hydrolysis	18.8	2810105	2810105	89.0	7.374	11.023	11.0	Pass

These outcomes showed that the suggested method is specific and sensitive for quantifying Vorasidenib.

Conclusion

The developed method is simple, precise, accurate and reliable for the estimation of Vorasidenib in Pharmaceutical dosage form. The %RSD of all results is less than 2% that shows high degree. Hence, the proposed method is specific and cost-effective and can be used for routine quantitative analysis of Vorasidenib in its Pharmaceutical dosage form.

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Conflict of Interest

The authors declare that no conflict of interest.

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