

## ESTABLISHMENT AND VALIDATION OF A RELIABLE RP-HPLC METHOD FOR STABILITY TESTING OF VISMODEGIB IN PHARMACEUTICAL DOSAGE FORMS

Uzma Tahniyath\*<sup>1</sup> and Aftab Azher Qureshi<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Analysis and Quality Assurance, St. Pauls College of Pharmacy, Hyderabad, Telangana, Ranga Reddy District, India.

<sup>2</sup>Bachelor of Pharmacy, St. Pauls College of Pharmacy, Hyderabad, Telangana, Ranga Reddy District, India.

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\*Corresponding Author: Uzma Tahniyath

Department of Pharmaceutical Analysis and Quality Assurance, St. Pauls College of Pharmacy, Hyderabad, Telangana, Ranga Reddy District, India.

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### ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Vismodegib by RP- HPLC technique. Chromatographic conditions used are stationary phase ODS C18 (250mm\*4.6mm3.6μ), Mobile phase 0.01N KH<sub>2</sub>PO<sub>4</sub>: Acetonitrile in the ratio of 65:35 and flow rate was maintained at 0.8ml/min, detection wave length was 264nm, column temperature was set to 30°C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R<sup>2</sup> value was found to be as 0.999. Precision was found to be 0.9 for repeatability and 0.8 for intermediate precision. LOD and LOQ are 0.33μg/ml and 0.99μg/ml respectively. By using above method assay of marketed formulation was carried out 99.86% was present. Degradation studies of Vismodegib were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Vismodegib.

**KEYWORDS:** HPLC Vismodegib, Method development, ICH Guidelines.

### INTRODUCTION

Vismodegib is used for the treatment of adults with metastatic basal cell carcinoma, or with locally advanced basal cell carcinoma that has recurred following surgery or who are not candidates for surgery, and who are not candidates for radiation.<sup>[1]</sup> Vismodegib is a crystalline free base with apKa (pyridinium cation) of 3.8, appearing as a white powder. Its

molecular weight is 421.30 g/mol and the partition coefficient (log P) is 2.7. Vismodegib exhibits pH dependent solubility and it has been classified as a Class 2 molecule under the Biopharmaceutics Classification System (BCS).<sup>[2]</sup> Vismodegib is chemically known as 2-Chloro-N-(4-chloro-3-(pyridine-2-yl) phenyl)-4- (methyl sulfonyl) benzamide and its molecular formula C<sub>19</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>S (Figure 1). The mechanism of action of Vismodegib binds to and inhibits smoothened, a transmembrane protein involved in Hedgehog signal transduction.<sup>[2]</sup> In this present work, a new sensitive and rugged RP-HPLC method was developed for the determination of Vismodegib in bulk, and this method was validated according to FDA and ICH guidelines.

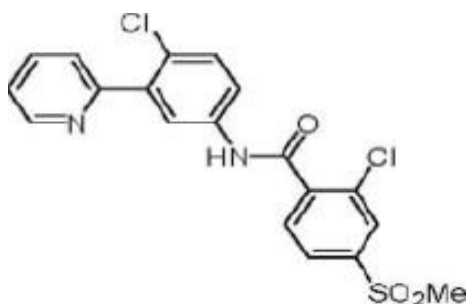


Figure 1: Chemical structure of Vismodegib.

## EQUIPMENTS AND CHEMICALS

### • Materials

Vismodegib pure drugs (API), Combination Vismodegib tablets (Evridge) Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

### Equipment and Apparatus used

1. HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Vismodegib solutions.

## METHOD DEVELOPMENT

Based on drug solubility and PKa Value following conditions has been used to develop the method estimation of Vismodegib.

### Optimized Method

#### Optimized Chromatographic Conditions

**Column:** ODS C18 250mm x 4.6 mm, 3.6μ.

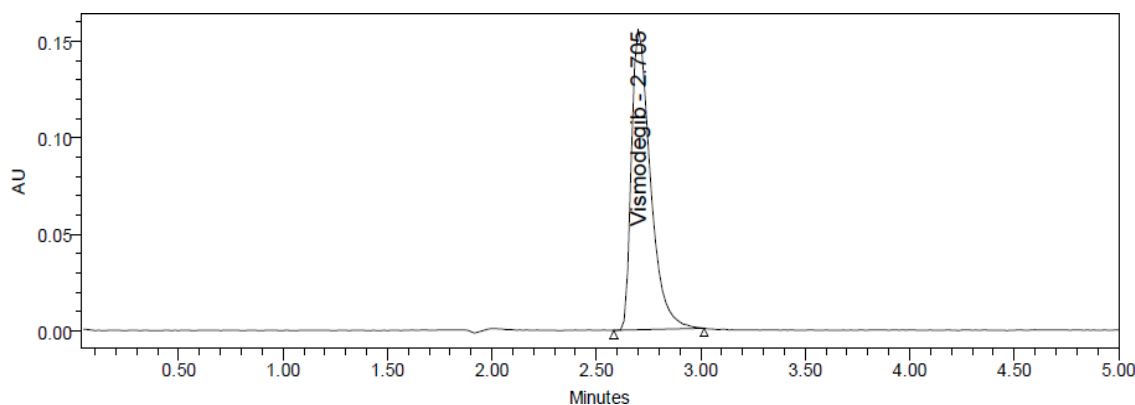
**Mobile phase:** 0.01N KH<sub>2</sub>PO<sub>4</sub>: Acetonitrile (65:35)

**Flow rate:** 0.8 ml/min

**Detector:** PDA 236nm

**Temperature:** 30°C

**Injection Volume:** 10μL



**Fig. 2: Optimized chromatogram -3.**

**Observation:** All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

**Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and buffer taken in the ratio of 50:50

**Preparation of Standard stock solutions:** Accurately weighed 75mg of Vismodegib transferred 50ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1500µg/ml of Vismodegib).

**Preparation of Standard working solutions (100% solution):** 1ml of Vismodegib from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (150µg/ml of Vismodegib).

**Preparation of Sample stock solutions:** 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1500 µg/ml of Vismodegib).

**Preparation of Sample working solutions (100% solution):** 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (150µg/ml of Vismodegib)

#### Preparation of buffer: Buffer

**0.1%OPA Buffer:** 1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water. **0.01N KH<sub>2</sub>PO<sub>4</sub>**

**Buffer:** Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.5 with dil. Orthophosphoric acid solution.

#### Validation

- Method validation: The developed method was validated as per FDA and ICH guidelines by evaluating Precision (Repeatability and Reproducibility) linearity, accuracy, degradation and robustness.
- Specificity: The ICH guidance defines specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present”<sup>[4]</sup>. Forced degradation study was conducted by exposing drug substance sample to various stress conditions. Stressed samples were analyzed, active peak was checked for the retention time, peaks interference and purity.
- Precision: Precision is defined as the measure of how close the data values are to each other for a number of measurements under the same analytical conditions<sup>[5]</sup> in precision analysis, system precision, method precision and intermediate precision have been carried out. The system precision was determined by analyzing standard solution in

six replicates, % RSD of area counts of Vismodegib peak was calculated. In method precision, six preparations of 100% test concentration against standard solution were analyzed. Intermediate precision was performed by different analyst on different day using different column. Overall RSD for assay between the two precision sets of data was calculated.

- **Linearity:** Linearity is defined as the ability to obtain test results which are directly proportional to the concentration of analyte in the sample. For the establishment of linearity, a minimum of 5 different concentrations is recommended<sup>[4]</sup>. From the standard stock solution, a series of solutions were prepared at concentration levels ranging from 0.012 mg/mL to 0.120 mg/mL. The peak area response of solutions at all levels in triplicate were measured. The peak response versus concentration data was treated by linear regression analysis and the linearity of response for Vismodegib was determined by calculating correlation coefficient.
- **Accuracy:** Accuracy is the measure of how close the experimental value is to the true value<sup>[6]</sup>. For the determination of accuracy, the standard addition method was applied. In this study known amount of active substance was spiked in sample solvent at three different levels in triplicate. Accuracy has been performed at about 50%, 100% and 150% of sample target concentration. The samples were analyzed by the proposed method and the amount of Vismodegib recovery was calculated by this formula:

$$\% \text{ Recovery} = \frac{\text{mg found}}{\text{mg added}} \times 100$$

- **Robustness:** Robustness of the method was investigated by varying the instrumental conditions such as flow rate ( $\pm 0.2$  mL/min), mobile phase composition ( $\pm 10\%$  absolute) and column temperature ( $\pm 5^\circ\text{C}$ ). System suitability criteria of the standard solution were checked at each minor variable condition. The retention time (RT), USP tailing factor, theoretical plate counts and % RSD of area counts of Vismodegib from standard solution for each set of data was calculated.
- **Forced degradation:** Force degradation or stress testing includes four main degradation mechanisms: thermal, acid/base hydrolysis, oxidative, and photolytic degradation. Selecting suitable reagents and length of exposure can achieve the preferred level of degradation. Over stressing a sample may lead to the formation of secondary degradants that would not be seen in formal shelf-life stability studies and under-stressing may not serve the purpose of stress testing.<sup>[7]</sup> Therefore, it is necessary to control the degradation to a desired level.
- **Thermal stress:** In thermal stress, solid drug substances and drug products should be exposed to heat. It is recommended that the effect of temperature be studied in  $10^\circ\text{C}$  increment above that for routine accelerated testing, and humidity at 75% relative humidity or greater.<sup>[8]</sup> The heating time can be increased if there is no significant degradation observed in initial study. By increasing the temperature, the rate of reaction also tends to increase the production of degradation products. Thermal degradation was performed by treating the Vismodegib drug substance at  $40^\circ\text{C}/75\%$  RH for 14 days in an open container. Sample was diluted as per required concentration with sample solvent and mixed. The obtained chromatogram was analyzed for any degradation occurred during the process. The results are given in Table 2.
- **Acid/base hydrolysis:** In this stress study, the drug reacts with different pH conditions. In general, the drug substances are treated with different concentrations of Hydrochloric acid and Sodium hydroxide. If the reasonable degradation was not achieved, then higher concentration or longer duration time can be extended. After subjected to stress conditions, the samples should be neutralized with acid or base to avoid further degradation.

- Acidic and basic degradations were performed using 0.1 M HCl and 0.1 M NaOH. Added 5.0 mL to each stock and refluxed at 60°C for 5 hours. After stressing sample stocks were neutralized with respective solutions and further diluted with sample solvent as per required concentration. The obtained chromatograms were analyzed for any degradation occurred during the process and the results are given in Table 2.
- Oxidation stress: For oxidation stress, drug substances require free radical initiators for oxidation process. Oxidizing agents such as hydrogen peroxide, metal ions, oxygen and radical initiators can be used in oxidation stress. Different stress conditions may generate the same or different degradates. The type and extent of degradation depends on the functional groups of the drug molecule and the stress conditions <sup>[7]</sup>
- Peroxide degradation of Vismodegib was performed using 3% Hydrogen peroxide. Added 5.0 mL of 3% H<sub>2</sub>O<sub>2</sub> and kept in water bath at 60°C for 2 hours. After attained room temperature, diluted to volume and further diluted as per required concentration. This solution was injected immediately to avoid excess degradation. The results are given in Table 2.
- Photolytic degradation: In this study, the drug substances are exposed to light source. Some recommended conditions for photostability testing are described in ICH Q1B photostability Testing of New Drug Substances and Products. Samples of drug substance, and solid/liquid drug product, should be exposed to a minimum of 1.2 million lux hours and 200-watt hours per square meter light. The samples should be exposed to both white and UV light. Temperature control may be necessary to minimize the effect of temperature changes during exposure <sup>[8]</sup>. The presence of the C=C, C=O, Aryl chloride, C<sub>6</sub>H<sub>4</sub>Cl<sub>2</sub>, Nitroaromatic group, -C<sub>6</sub>H<sub>4</sub>NO<sub>2</sub>, a weak C-H bond, Sulphides, alkanes, polyenes, and phenol's chemical function groups in the drug molecules is usually necessary for the occurrence of photochemical reactions. <sup>[9]</sup>

### Assay Methodology

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae. Calculate the percentage purity of Vismodegib present in tablet using the formula:

### CALCULATION

$$\text{Spl area Std. Dil. Fac Avg. Wt of Tab Potency of Std Assay} = \frac{\text{-----} \times \text{-----}}{\text{L.C}} \times \text{Std area Spl. Dil. Fac}$$

Spl area – Sample Peak area Std area – Standard Peak area Std. Dil. Fac- standard dilution factor Spl. Dil. Fac- sample dilution factor Avg. Wt of Tab- average weight of tablet

L.C – label claim Potency of Std

### RESULTS AND DISCUSSIONS

#### System Suitability

A Standard solution of Vismodegib working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from Six replicate injections are within the range and Results were shown in table 1.

**Table 1: System Suitability Parameters.**

Peak Name: Vismodegib					
	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Vismodegib	2.705	1086373	4165	1.60
2	Vismodegib	2.717	1087779	4264	1.59
3	Vismodegib	2.742	1075524	4252	1.59
4	Vismodegib	2.750	1073838	4784	1.62
5	Vismodegib	2.755	1076799	4378	1.57
6	Vismodegib	2.755	1071205	4331	1.57
Mean			1078586		
Std. Dev.			6851.9		
% RSD			0.6		

- Precision**

**Repeatability:** Six working sample solutions of 150ppm are injected and the % Amount found was calculated and %RSD was found to be 0.7 and chromatogram was shown in fig.

**Table 2: Repeatability data.**

S. No.	Peak Area
1	1084986
2	1075847
3	1066733
4	1086424
5	1082762
6	1078892
AVG	1079274
STDEV	7281.2
%RSD	0.7

- Intermediate precision:** Five working sample solutions of 150ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.4.

**Table 3: Intermediate precision data.**

S. No.	Peak Area
1	991168
2	992052
3	982607
4	984659
5	986092
6	982160
AVG	986456
STDEV	4246.2
%RSD	0.4

**LINEARITY**

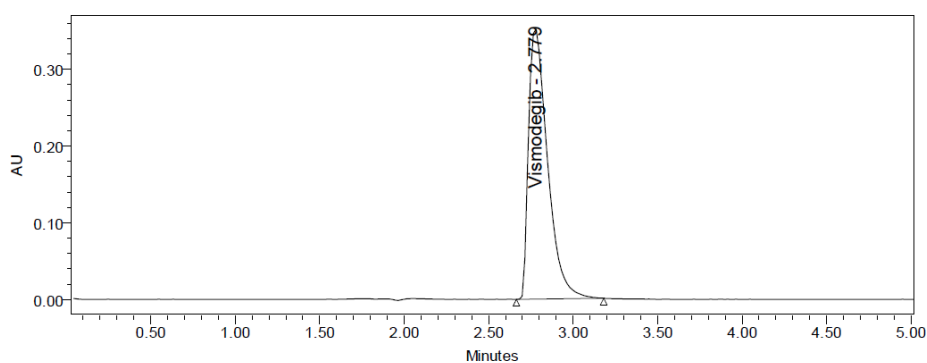
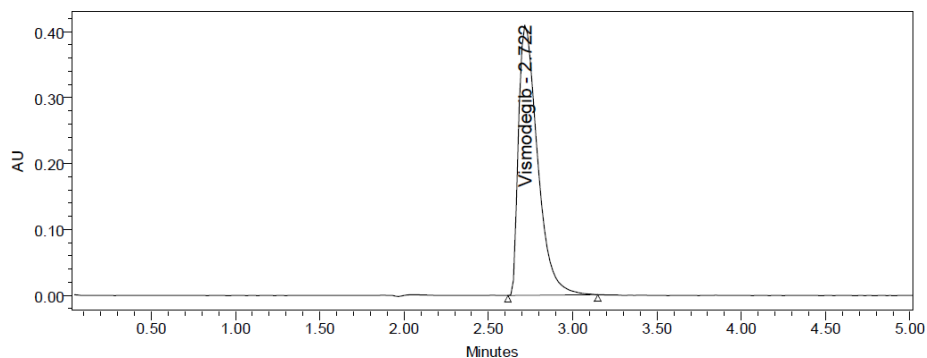
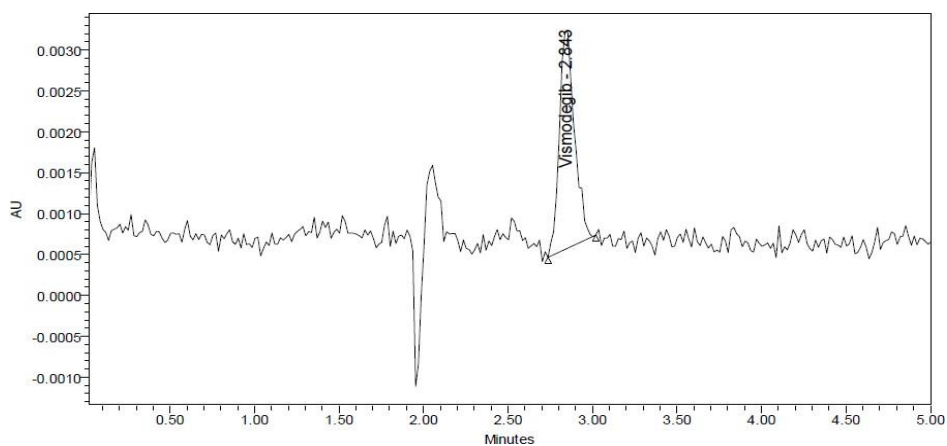
To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 37.5 ppm to 225ppm of Vismodegib. Plot a graph to concentration versus peak area. Slope obtained was 6765 Y-Intercept was 2312 and Correlation Co-efficient was found to be 0.999.

**Accuracy**

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and % Recovery was calculated as 100.16.

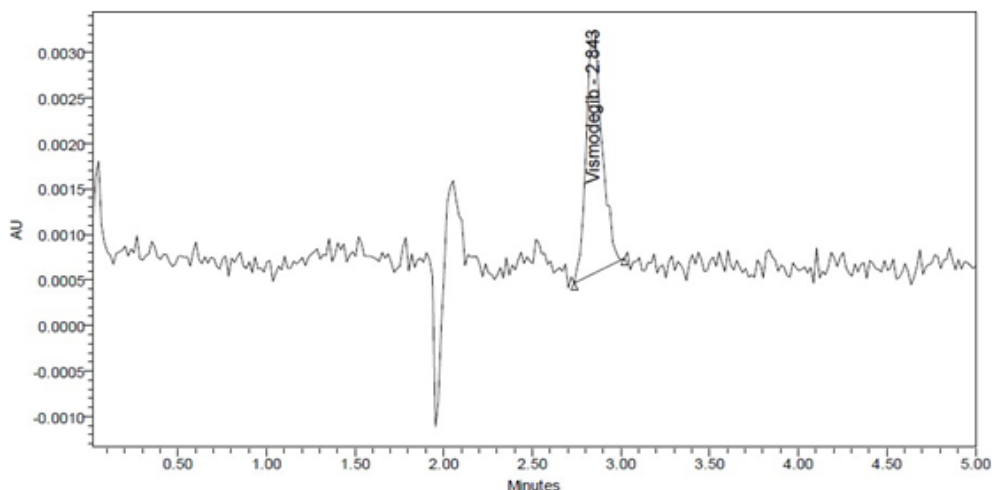
**Table 5: Accuracy data.**

% Level	Amount Spiked ( $\mu\text{g/mL}$ )	Amount recovered ( $\mu\text{g/mL}$ )	% Recovery	Mean % Recovery
50%	50	74.62	99.50	100.16%
	50	75.64	100.86	
	50	75.41	100.55	
100%	100	149.37	99.58	
	100	149.41	99.61	
	100	152.83	101.89	
150%	150	224.21	99.65	
	150	225.24	100.10	
	150	224.35	99.71	

**Fig. 3: Accuracy 50% Chromatogram.****Fig. 4: Accuracy 100% Chromatogram.****Fig. 5: Accuracy 150% Chromatogram.**

**LOD**

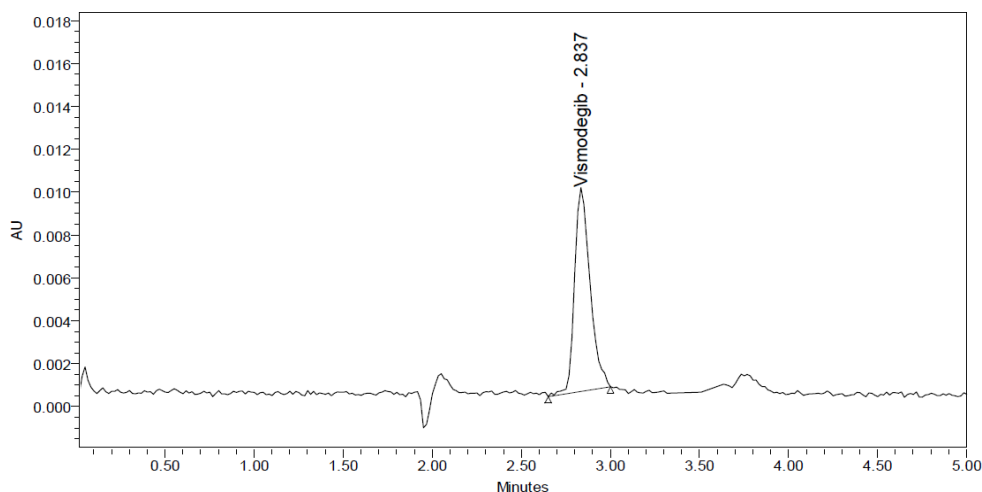
Detection limit of the Vismodegib in this method was found to be 0.33/ml.



**Fig. 6: LOD Chromatogram of Vismodegib.**

**LOQ**

Quantification limit of the Vismodegib in this method was found to be 0.99 $\mu$ g/ml.



**Fig. 7: LOQ Chromatogram of Vismodegib.**

**Robustness**

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. % RSD of the above conditions are calculated.

**Table 6: Robustness Data.**

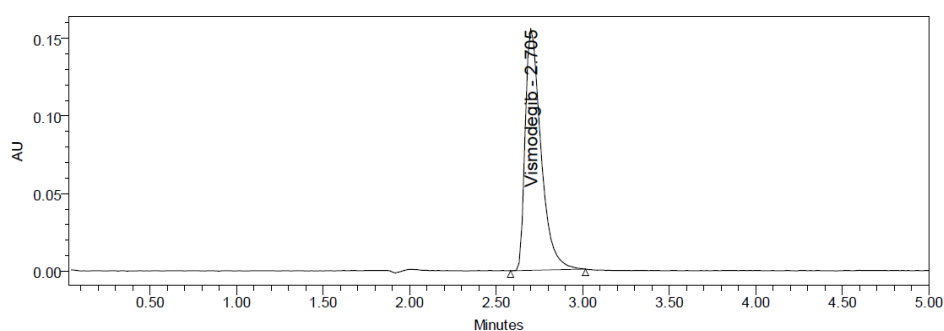
Parameter	% RSD
Flow Minus	1.4
Flow Plus	0.5
Mobile phase Minus	0.2
Mobile phase Plus	0.8
Temperature minus	0.4
Temperature plus	0.9

### ASSAY OF MARKETED FORMULATION

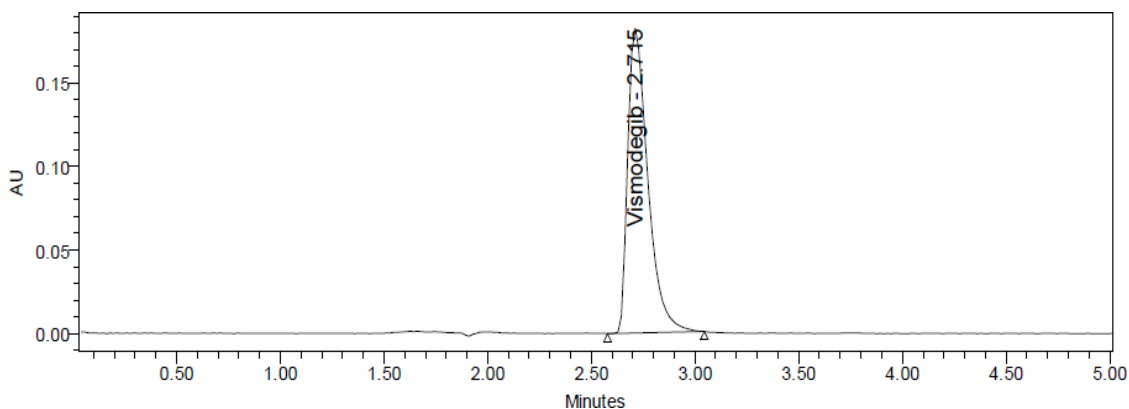
Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

**Table 7: Assay of Formulation.**

Sample No.	% Assay
1	100.39
2	99.55
3.	98.70
4.	100.53
5.	100.19
6.	99.83
AVG	99.86
STDEV	0.67
%RSD	0.7



**Fig. 8: Standard chromatogram.**



**Fig. 9: Assay Chromatogram.**

### Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

### Degradation procedure

**Oxidation:** To 1 ml of stock solution of Vismodegib 1 ml of 20% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added separately. The solutions were kept for 30 min at 60<sup>0</sup>c. For HPLC study, the resultant solution was diluted to obtain (150ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

### Acid Degradation Studies

To 1 ml of stock solution Vismodegib 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 1c. The resultant solution was diluted to obtain (150ppm) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

### Alkali Degradation Studies

To 1 ml of stock solution Vismodegib 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60<sup>0</sup>c. The resultant solution was diluted to obtain (150ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

### Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105<sup>0</sup>c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (150ppm) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

### Photo Stability studies

The photochemical stability of the drug was also studied by exposing the (1500ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200-Watt hours/m<sup>2</sup> in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (150ppm) solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

### Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60<sup>0</sup>c. For HPLC study, the resultant solution was diluted to (150ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Table 8: Degradation Data of Vismodegib.**

S. No.	Degradation Condition	Peak Area	% Recovery	% Drug Recovery
1	Acid	1011547	93.60	6.40
2	Alkali	1025995	94.93	5.07
3	Oxidation	1004391	92.93	7.07
4	Thermal	1022834	94.64	5.36
5	UV	1069674	98.98	1.02
6	Water	1072765	99.26	0.74

Chromatographic conditions used are stationary phase ODS C18 (250mm\*4.6mm5µ), Mobile phase 0.01N KH<sub>2</sub>PO<sub>4</sub>: Methanol in the ratio of 65:35 and flow rate was maintained at 0.8ml/min, detection wave length was 264nm, column temperature was set to 30°C and diluent was mobile phase. Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150 % levels, R<sup>2</sup> value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 0.7 for intermediate precision. LOD and LOQ are 0.33µg/ml and 0.99µg/ml respectively. By using above method assay of marketed formulation was carried out 100.16% was present. Degradation studies of Vismodegib were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Vismodegib.

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