

# Quantification and Stability Indicating Method Development and Validation of Vismodegib in Bulk and Pharmaceutical Dosage Form by Ultra Performance Liquid Chromatography

## ABSTRACT:

**Background:** Vismodegib (VMD) is a drug of choice for the treatment of basal-cell carcinoma. Present studies carried out to estimate VMD by RP-UPLC technique and to develop a simple, precise, accurate method for routine analysis.

**Study design:** Quantification and Stability Indicating Method Development and Validation of VMD in Bulk and Pharmaceutical Dosage Form by Ultra Performance Liquid Chromatography was planned and executed.

**Place and Duration of Study:** Instrumental Analysis Lab, Department of Pharmaceutical Analysis, Joginpally B.R. Pharmacy College, Hyderabad-500075, Telangana, India, between July 2020 and December 2020.

**Methods:** For this purpose Chromatographic conditions used were stationary phase ODS C18 (100mm x 2.1 mm, 1.8 $\mu$ ), Mobile phase 0.01N KH<sub>2</sub>PO<sub>4</sub>: Acetonitrile in the ratio of 50:50 and flow rate was maintained at 0.3ml/min, detection wave length was Acquity TUV 254nm, column temperature was set to 30°C and diluent was mobile phase, Conditions were finalized as optimized method.

**Results:** System suitability parameters were studied by injecting the standard six times. Linearity study was carried out between 25% to 150 % (25 - 150 ppm) levels, R<sup>2</sup> value was found to be as 0.9992. Precision was found to be 0.6 for repeatability and 0.4 for intermediate precision. LOD and LOQ are 0.33 $\mu$ g/ml and 0.99 $\mu$ g/ml respectively and results were well under the acceptance criteria.

**Conclusion:** By using above method assay of marketed formulation was carried out and was found 99.86%. Degradation studies of VMD were done, in all conditions purity threshold was more than purity angle and within the acceptable range. The developed method was simple and can be used for routine analysis.

**Key words:** UPLC, Vismodegib (VMD), Method development, ICH Guidelines.

## 1. INTRODUCTION

VMD IUPAC name is 2-chloro-N-(4-chloro-3-pyridin-2-ylphenyl)-4-methyl sulfonyl benzamide (Fig. 1). VMD is an orally bioavailable molecule with potential antineoplastic activity, acts as Hedgehog antagonist, and targets the Hedgehog signaling pathway. It blocks the activities of the Hedgehog-ligand cell surface receptors and suppresses the Hedgehog signaling. The Hedgehog signaling pathway plays a vital role in tissue growth and repair. The Hedgehog pathway plays a crucial role during embryogenic development and has limited activity in most adult tissues, with the exception of hair, skin and stem cells [1-8].

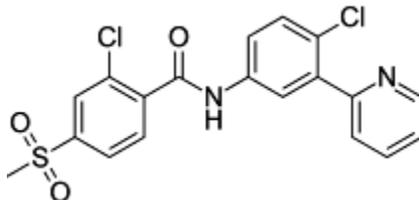


Fig. 1. Structure of VMD

After a detailed study, Literature delineate that few analytical methods were reported and available for quantification of VMD, by using HPLC [9-10] and LCMS/MS [11-13]. No methods were available on UPLC method, hence decided to be developed and validate this method as per ICH norms [14-16].

## 2. MATERIALS AND METHODS

### 2.1. Materials

VMD standard API obtained from Spectrum lab Private Ltd., Erivedge (Genentech) tablet dosage forms, distilled water (milli-Q), Acetonitrile, phosphate buffer and potassium dihydrogen phosphate buffer. All chemicals, HPLC grade, Merck, are purchased from local distributor.

### 2.2. Instruments

UPLC instrument used was of WATERS UPLC 2965 SYSTEM with Auto Injector and Acquity TUV 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 of VMD. Sonicator (Ultrasonic sonicator), P<sup>H</sup> meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump (Welch) are the other instruments used for this study.

### 2.3. Analytical methodology

#### 2.3.1. Preparation of Standard and Sample stock solutions

Accurately weighed 75mg of VMD transferred 50ml and volumetric flasks, 3/4<sup>th</sup> of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1500µg/ml of VMD).

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 UPLC filters and labeled as Standard stock solution (1500 µg/ml of VMD).

#### 2.3.2. Preparation of Standard and Sample working solutions (100 % solution)

From the above Standard and Sample stock solutions, 1ml of VMD was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (150µg/ml of VMD).

#### 2.3.3. Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of VMD are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of VMD.

#### 2.3.4. Accuracy preparations

From the formulation solution take 0.5ml, 1ml, 1.5ml, was transferred to 10 ml volumetric flask and make up the volume to get 50% 100% and 150% solution concentrations.

### 2.4. Validation Procedure[16]

The analytical method was validated as per ICH Q2 (R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ) and forced degradation.

#### 2.4.1. System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

#### 2.4.2. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and the amount of the drug present, percentage of recovery and related standard deviation were calculated.

#### 2.4.3. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the present method was assessed in terms of repeatability, intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

#### 2.4.4. Linearity and range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample within a given range. The six series of standard solutions were injected for assessing linearity range. The calibration curve was plotted using peak area with concentration of the standard solution and the regression equations were calculated.

#### 2.4.5. LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were separately determined based on the calibration curve. The LOD and LOQ of VMD determined by injecting progressively low concentrations of standard solutions by using the developed method. The LOD and LOQ were calculated as  $3.3s/n$  and  $10s/n$  respectively as per ICH guidelines, where  $s/n$  indicates signal-to-noise ratio.

#### 2.4.6. Stress degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1A (R2). The degradation peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions (acid, alkali, oxidation, thermal, UV, water) to obtain the degradation of about 20%.

#### 2.4.7. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the UPLC system and altered chromatographic conditions such as Flow minus, Flow plus, mobile phase minus, mobile phase plus, temperature minus and temperature plus. The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

#### 2.4.8. Statistical analysis

The data obtained were analysed by Graph pad prism software version 9. The data is subjected to regression analysis to obtain the line of equation in linearity studies.

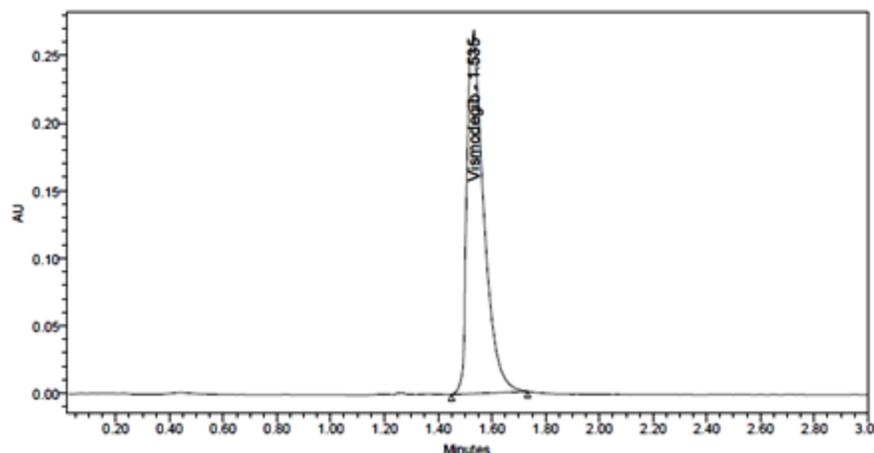
### 3. METHOD DEVELOPMENT

#### 3.1. Optimized method

Trials were performed for the method development and the best peak with least fronting factor was found to be with  $RT = 1.535$  min. for VMD Optimized chromatographic conditions were shown in Table 1 and optimized chromatogram was shown in figure 2.

**Table 1. Optimized Chromatographic conditions**

Parameter	Content
Column	STD BEH C <sub>18</sub> 100mm x 2.1 mm, 1.8 $\mu$ .
Mobile Phase	A mixture of Methanol:KH <sub>2</sub> PO <sub>4</sub> taken in the ratio 50:50%v/v with a pH 7.4
Flow Rate	0.3 ml/min
Temperature	30 <sup>o</sup> C
Injection Volume	10 $\mu$ l
Wavelength	Acquity TUV 254nm



**Fig. 2: Optimized chromatogram**

### 3.2. System Suitability

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. System suitability parameters were shown in table 2.

**Table 2. System suitability parameters for VMD**

S. No.	Retention time (R <sub>t</sub> )	Theoretical plates (N)	Tailing factor (T)
1	1.57	2739	1.54
2	1.601	2558	1.49
3	1.633	2895	1.51
4	1.638	2902	1.53
5	1.655	2929	1.53
6	1.677	3066	1.51
<b>AVG ± %RSD</b>	1.63 ± 2.4	2848.17 ± 6.2	1.52 ± 1.2
<b>S.D</b>	0.04	176.18	0.02

Where AVG=Average, %RSD=relative standard deviation, S.D=standard deviation (n=6)

## 4. METHOD VALIDATION

### 4.1. Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.62%. Recovery study values were shown in Table 3.

**Table 3. Recovery studies for VMD**

% Concentration	VMD					
	Trail	Peak area	ADD ppm	x-std ppm	%recovery	AVG ± %RSD
50 %	I	1811135	75	74.31	99.08	99.04 ± 0.37
	II	1815463	75	74.85	99.80	
	III	1812750	75	74.51	99.35	
100 %	I	2399749	150	148.16	98.77	99.20 ± 1.02
	II	2396163	150	147.71	98.47	
	III	2418632	150	150.53	100.35	

150 %	I	3017536	225	225.67	100.30	100.25 ± 0.12
	II	3014127	225	225.25	100.11	
	III	3018237	225	225.76	100.34	
			<b>AVG ± %RSD</b>		99.62 ±0.73	
			<b>SD</b>		0.72	

Where AVG=Average, %RSD=relative standard deviation, S.D=standard deviation (n=6)

#### 4.2. Precision

Six working sample solutions of 150ppm are injected and the % Amount found was calculated. The Precision % RSD value obtained as 0.6 % and Intermediate precision value obtained as 0.4 % respectively. As the limit of Precision was less than “2” the system precision was passed in this method. System precision values were shown in Table 4.

**Table 4. System precision table of VMD**

S.No.	Peak area of VMD	
	Peak area	Day day Precision
1	1023018	1015988
2	1021641	1014129
3	1029838	1018620
4	1035222	1006613
5	1025265	1014711
6	1020254	1018866
<b>AVG ± %RSD</b>	1025873±0.6	1014821±0.4
<b>SD</b>	5676.8	4472.6

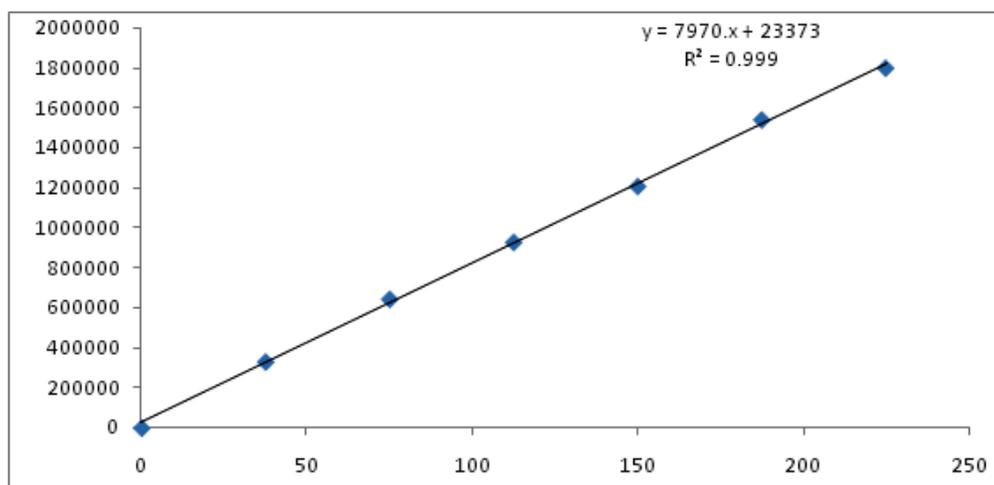
Where AVG=Average, %RSD=relative standard deviation, S.D=standard deviation (n=6)

#### 4.3. Linearity

Six linear concentrations of VMD within the vary of 25 - 150 ppm were injected in a duplicate manner. The slope and intercept value for calibration curve of VMD was found to be  $y = 7970.1x + 23373$  ( $R^2=0.9992$ ) Linearity plot was shown in Figure 3, Linearity table shown in Table 5.

**Table 5. Linearity table of VMD**

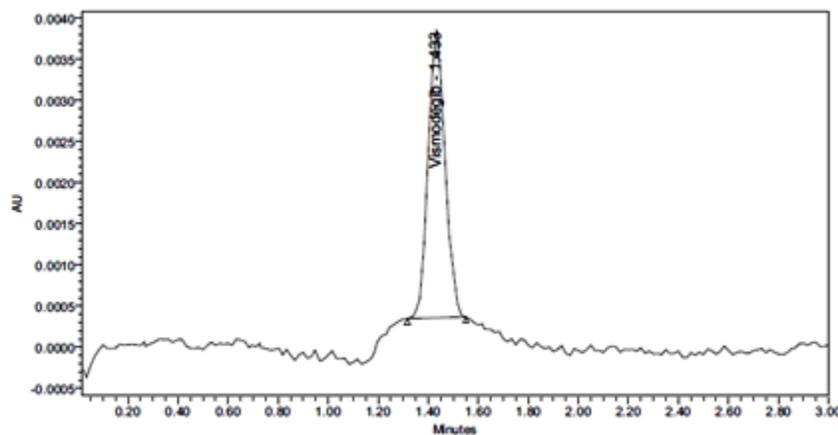
Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	37.5	329888
50	75	642854
75	112.5	926754
100	150	1206099
125	187.5	1538247
150	225	1796209



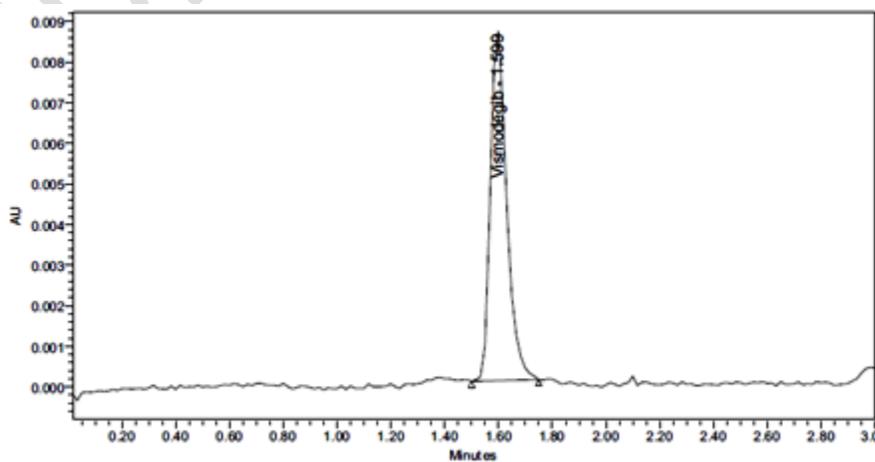
**Fig. 3. Linearity curve of VMD**

#### 4.4. LOD and LOQ

LOD and LOQ were estimated from the signal-to-noise ratio. Detection limit of the VMD in this method was found to be  $0.33\mu\text{g/ml}$ . Quantification limit of the VMD in this method was found to be  $0.99\mu\text{g/ml}$ . LOD and LOQ Chromatograms were shown in figure 4 & 5 respectively.



**Fig. 4. LOD Chromatogram of VMD**



**Fig. 5. LOQ Chromatogram of VMD**

#### 4.6. 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 is calculated. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit. Robustness data were shown in table 6.

**Table 6. Robustness data of VMD**

S. No	Condition	% RSD of VMD
1	Flow rate (-) 0.2 ml/min	1.1
2	Flow rate (+) 0.4 ml/min	0.9
3	Mobile phase (-) 45B:55A	0.4
4	Mobile phase (+) 55B:45A	0.4
5	Temperature (-) 25°C	0.8
6	Temperature (+) 35°C	0.5

#### 4.7. Degradation Studies

Degradation studies in all conditions like Acid, Alkali, Oxidation, Thermal, UV and Water 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 values were shown in table 7.

**Table 7. Degradation Data of VMD**

S.NO	Degradation Condition	Peak Area	% Recovery	% Drug Degraded
1	Acid	992111	96.82	3.18
2	Alkali	981497	95.79	4.21
3	Oxidation	943844	92.11	7.89
4	Thermal	988612	96.48	3.52
5	UV	983164	95.95	4.05
6	Water	986766	96.30	3.70

#### 4.8. 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. Average % Assay obtained was 100.12%. Assay Data of Marked Formulation were shown in table 8.

**Table 8. Assay of Formulation**

Sample No	%Assay
1	99.84
2	99.71
3.	100.51
4.	101.03
5.	100.06
6.	99.57
AVG	100.12
SD	0.55
%RSD	0.6

Where AVG=Average, %RSD=relative standard deviation, S.D=standard deviation (n=6)

## 5. CONCLUSION

The present study describes new and easy RP-UPLC methodology for the estimation of VMD. The strategy valid was found to be accurate and precise. Thus the projected studies may be used for quantification of VMD in bulk and pharmaceutical dosage form and can be adopted in regular Quality control test in Industries.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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