RP-HPLC METHOD FOR THE QUANTIFICATION OF VISMODEGIB IN FORMULATIONS

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Vismodegib in tablet dosage form. Isocratic elution at a flow rate of 1 mL/min was employed on a symmetry Chromosil C18 (250 mmx4.6 mm I.D., 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: water: Acetonitrile 55:25:20 by volume. The UV detection wavelength was 236 nm and 20µl sample was injected. The retention time for Vismodegib was 8.29mins. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Vismodegib in tablet dosage form and bulk drug.

Key Words: Vismodegib, RP-HPLC, UV detection, recovery, precise, 236 nm

INTRODUCTION

Vismodegib (figure.1) is a first-in-class small-molecule inhibitor of the Hedgehog signaling pathway and is currently in clinical development for treatment of various cancers. In a previous phase 1 clinical trial in patients with solid tumors, Vismodegib was well tolerated and promising efficacy in advanced basal cell carcinoma was observed.

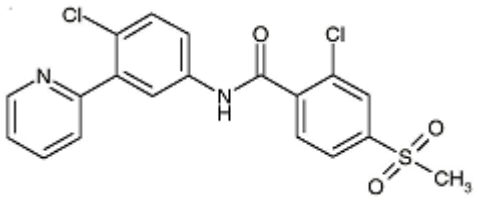

Figure.1 Structure of Vismodegib

EXPERIMENTAL

Materials

Working standard of Vismodegib was obtained from well reputed research laboratories. Acetonitrile, Methanol, water was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. (250mm×4.6mm,i.d.,5µm pariclesize) Electronic balance-DENVER (SI-234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. Analytical column chromosil C18 (250 mm X 4.6 mm.,i.d.,5µm particle size) was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of Vismodegib were scanned in the range of 200 -400 nm against mobile phase as a blank. Vismodegib showed maximum absorbance at 236 nm. So the wavelength selected for the determination of vismodegib was 236 nm.

Chromatographic conditions

The mobile phase consisted of a Methanol: water: Acetonitrile 55:25:20 by volume. Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 mL/min. Detection was performed at 236 nm with 10 mins runtime.

Standard and sample solutions

10 mg of Vismodegib reference substance was accurately weighed and dissolved in 10 mL mobile phase in a 10 mL volumetric flask to obtain 1000 µg/mL concentrated solution. From standard solution by the serial dilution, required concentrations for the calibration curve was prepared.

Twenty capsules of Vismodegib (Erivedge) were powdered and weighed. A quantity of powder equivalent to 10 mg of Vismodegib is transferred in to 10mL light

resistant flask and made upto the required volume by using mobile phase. Pipetted out 1mL of resulting solution into the 10 mL volumetric flask and made upto the required volume by using mobile phase and sonicated for 15 min, filtered through 0.45µm membrane filter. From this 75µg/mL sample solution was prepared.

Optimized chromatographic conditions

Chromatographic conditions as optimized above are shown in Table.1. These optimized conditions were followed for the determination of Vismodegib in bulk samples and its tablet Formulations. The chromatograms of standard and blank are shown in Figure.2 and Figure. 3 respectively.

Table 1: Optimized chromatographic conditions for the estimation of Vismodegib

Mobile phase	**:** Methanol: Water: Acetonitrile: 55:25:20 (v/v/v)
Pump mode	**:** Isocratic
pH	**:** 6.1(adjusted with 0.1% OPA)
Diluent	**:** Mobile phase
Column	**:** Chromosil C18 column (250mm X 4.6 mm i.d., 5µm particle size)
Column Temp	**:** Ambient
Wavelength	**:** 236nm
Injection Volume	**:** 20µL
Flow rate	**:** 1.0mL/min
Run time	**:** 10mins
Typical t_R of Vismodegib	**:** 8.29mins

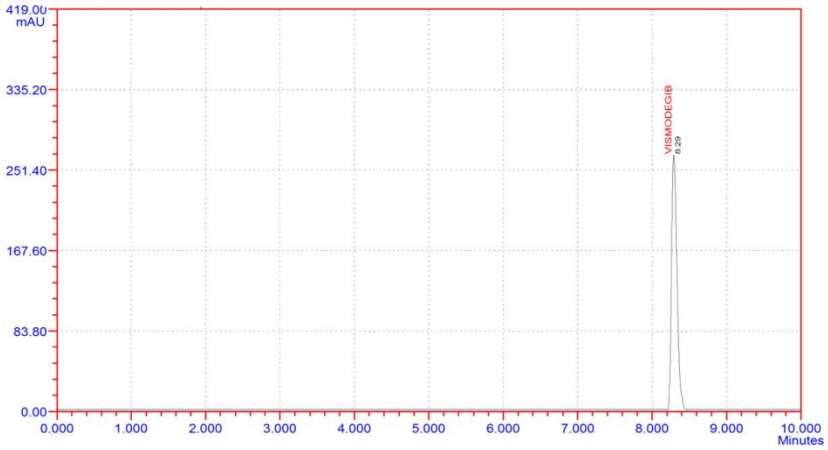

Figure.2 Chromatogram of Standard solution

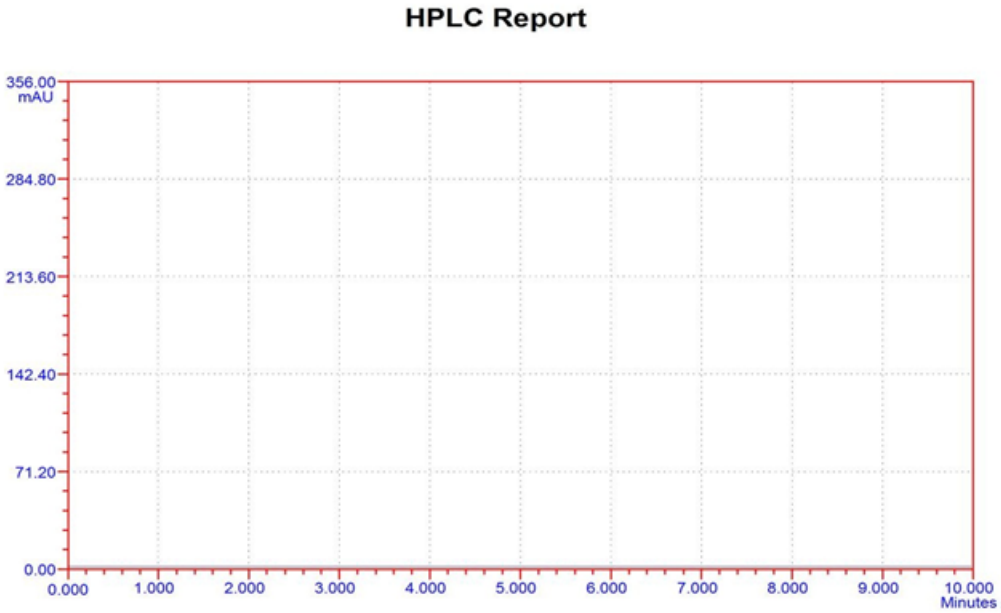

Figure 3 Chromatogram of the blank(no peak)

Method validation

Method validation was performed following ICH specifications for specificity, linearity, accuracy, precision and robustness.

SPECIFICITY :

The specificity of method was performed by comparing the chromatograms of blank ,standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are given in Table 2 .

Table2: Specificity study of Ruxolitinib

Name of the solution	Retention Time in Mins
Blank	No peaks
Vismodegib	8.29mins

Linearity

The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 8114.3 X-24439 (correlation coefficient= 0.999). Linearity values are given in Table: 3.

Table.3 Linearity Results of Vismodegib

Level	Concentration of Vismodegib in µg/mL	peak area
Level 1	25	186432
Level 2	50	386421
Level 3	75	564057
Level 4	100	786843
Level 5	125	989765
Level 6	150	1199879
	Slope Intercept Correlation Coefficient	8114.3 -24439 0.9992

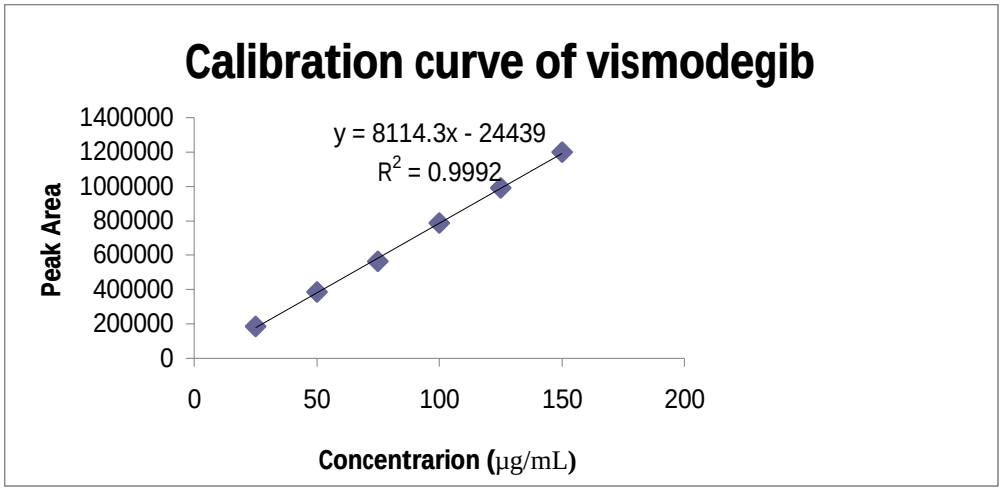

Figure 4. Linearity Graph of Vismodegib

Precision:

Repeatability of the method was checked by injecting replicate injections of 75 µg/mL of the solution for six times on the same day and %RSD for intraday was 0.50% .For inter day precision, six replicate injections of standard solution was injected on third day of sample preparation and the % RSD was 0.13%.Results are given in Table 4.

Table 4 : Precision parameters of Vismodegib

Injection	Concentration(µg/mL)	Inter day	Intra day
1	75	569784	563942
2		570125	565864
3		568975	569832
4		570241	562102
5		571021	567463
6		571076	563422
		%R.S.D =0.138	%R.S.D =0.506

Intermediate precision or Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions (75 µg/mL**)** which were prepared and analyzed by different analysts on three different days. Ruggedness is also expressed in terms of percentage relative standard deviation. Results are given in table 5.

Table 5 : Ruggedness results of Vismodegib

Sample	Conc. (in ppm)	Injection No.	Peak Areas	Intermediate precision %RSD (Acceptance criteria ≤ 2.0%)
Vismodegib	75	1	568812	0.37
		2	567901	
		3	568903	
		4	569098	
		5	563427	
		6	568120	

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 2.5 µg/mL dilution Peak was not clearly

observed, based on which 2.5 µg/mL is considered as Limit of Detection and Limit of Quantification is 6.25 µg/mL .

Table 6: LOD and LOQ results of vismodegib

Parameter	Measured Value
Limit of Quantification	6.25 µg/mL
Limit of Detection	2.5 µg/mL

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. Results are given in table 7.

Table 7: Robustness results of Vismodegib

Condition	%Assay	%Difference
Unaltered	100	
Wavelength at 238nm	101.09	1.09
Wavelength at 234nm	100.64	0.64
Mobile phase:		
Methanol(60):ACN (10): Water (25) v/v	100.16	0.16
Methanol(45):ACN (30): Water (25) v/v	101.08	1.01
pH of buffer at 5.9	101.07	1.07
pH of buffer at 6.3	100.16	0.16

Accuracy:

Recovery test was performed at 3 different concentrations i.e. 75µg/mL , 100µg/mL and 125µg/mL . Results are given in table.8.

Table.8 Recovery results of Vismodegib

% Recovery	Target Conc., (µg/mL)	Spiked conc., (µg/mL)	Final Conc. (µg/mL)	Conc., Obtained	% of Recovery
50%	50	25	75	74.63	99.50
	50	25	75	76.48	101.97
	50	25	75	77.07	102.76
100%	50	50	100	99.85	99.85
	50	50	100	101.62	101.62
	50	50	100	101.65	101.65
150%	50	75	125	124.27	99.41
	50	75	125	126.12	100.89
	50	75	125	126.98	101.58

Stability test

To perform the Stability test the standard solution of 75ppm was stored at ambient temperature (±10^{0}C) for two days. After this, these storage solutions and freshly prepared solution were tested with proposed method. It is noticed that assay of these results were not less than 98%. The results of stability test were shown in Table.9.

Table 9: Stability results

S. No	Concentration ppm	Standard solution	Area	%of Assay
1	75	Fresh	564057	100.0
2	75	Stored at +10^{o}c 1 2 3	 564032 563970 563825	 99.99% 99.98% 99.95%

System suitability

System suitability was studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in Table.10.

Table 10:System suitability parameters

Parameter	Tailing factor	Theoretical plates
Specificity study	1.22	73958
Linearity study	1.53	73043
Precision study	1.48	73527

Assay of Formulation of Vismodegib:

Vismodegib (Erivedge- 50 mg), 20 tablets were weighed and calculated the average weight. Accurately weighed and transferred the sample equivalent to 10mg of Vismodegib into a 10mL volumetric flask. Diluent is added and sonicated to dissolve it completely and made olume up to the mark with diluents. Mixed well and filtered through 0.45µm membrane filter paper. Further pipetted 1mL of the above stock solution into a 10mL volumetric flask and diluted up to mark with diluents and finally 75µg/mL was prepared. Mixed well and filtered through 0.45µm membrane filter paper. An aliquot of this solution was injected into HPLC system. Peak area of Vismodegib was measured for the determination

Table.11: Assay result

S.NO	Tablet	Dosage	Sample conc µg/mL	Sample estimated	% of Drug Estimated in Tablet
1	Erivedge	150 mg	75	74.505	99.34

CONCLUSION

The proposed method for the assay of Vismodegib in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. The method can be used for the routine analysis of Vismodegib in its tablet dosage forms.

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