# 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 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: water: Acetonitrile 55:25:20 % (V/V). The UV detection wavelength was 236 nm and 20µl sample was injected. The retention time for Vismodegib was 8.29min. 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 (GDC-0449) 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. Since Vismodegib was highly bound to alpha-1-acid glycoprotein (AAG) and the pharmacokinetics (PKs) was nonlinear in patients, binding to AAG is perceived as an important determinant of total Vismodegib plasma concentrations.

Equilibrium dialysis (ED) is one of the most commonly used methods for protein binding assessment and allows for quantitative determination of bound and unbound drug concentrations in plasma. It can minimize the effect of non specific binding and does not require large plasma volumes that impede other alternative techniques such as ultra filtration and ultracentrifugation.

In the present study, a RED assay followed by a solid phase extraction high-performance liquid chromatography tandem mass spectrometry assay for the determination of unbound Vismodegib concentration in human plasma was developed and validated. The RED assay procedure involved the use of plasma: buffer mixed matrix. The described solid phase extraction high-performance liquid chromatography tandem mass spectrometry assay used to quantitate unbound Vismodegib concentrations offered advantages 1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved. doi:10.1016/j.jchromb.2011.05.0482120 Y. Deng et al. / J. Chromatogram. B 879 (2011) 2119–2126 over our previous reported plasma assay in that it was compatible with our RED assay procedure and offered greater sensitivity required to measure low unbound concentrations. The validated assays were used to support multiple clinical trials, including a dose escalation study in cancer patients a study to optimize the Vismodegib dosing regimen (data on file) and a healthy volunteer PK study.

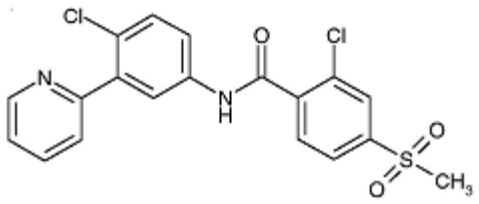



Figure.1 Structure of Vismodegib

**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. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

**Determination of wavelength of maximum absorbance**

The standard solutions of Vismodegibwere 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 equipment and conditions**

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisted of a Methanol: water: Acetonitrile: 55:25:20 (v/v). 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 min runtime.

**Standard and sample solutions**

A 10 mg amount of Vismodegib reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. From standard solution by the serial dilution, required concentrations including standard concentration of 150ppm was prepared.

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Vismodegib was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase was added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 75 ppm.

**Method validation**

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

**RESULTS AND DISCUSSION**

**System Suitability**

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the

analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

| 1 | **Mobile phase** | **Methanol: Water: Acetonitrile: 55:25:20(v/v)** |
|---|---|---|
| 2 | Pump mode | Isocratic |
| 3 | Column | Zodiac C18 column (250 X 4.6 mm, 5µ) |
| 4 | Column Temp | Ambient |
| 5 | Wavelength | 236 nm |
| 6 | Injection Volume | 20 µl |
| 7 | $P^H$ | 6.1 |
| 8 | Concentration | 75ppm |
| 9 | Retention Time | 8.29 |
| 10 | Run Time | 10min |
| 11 | Area | 564057 |
| 12 | Th. Plates | 73958 |
| 13 | Tailing Factor | 1.22 |
| 14 | Pump Pressure | 6.1 MPa |

**Table.1 System suitability parameters**

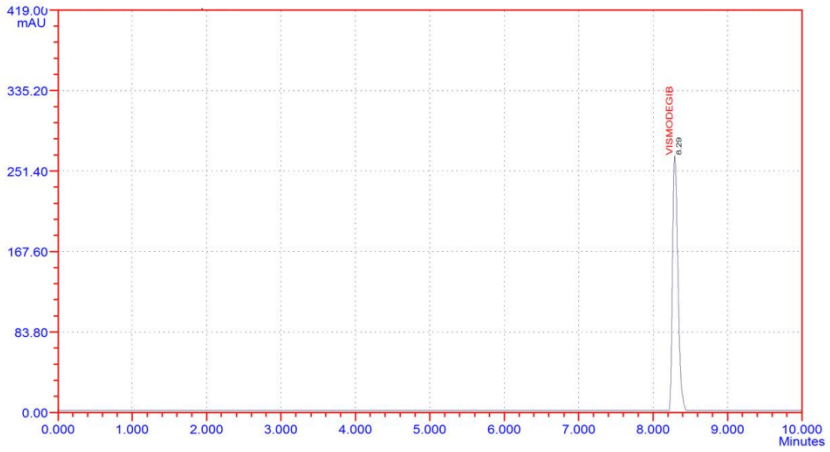


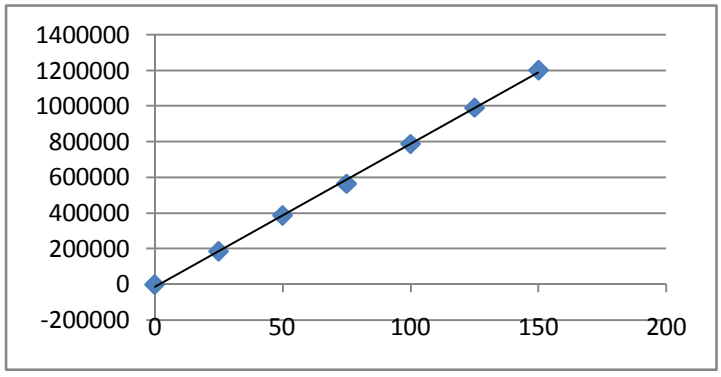

**Figure.2 Standard HPLC chromatogram of Vismodegib**

**Range of linearity**

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 25, 50, 75, 100, 125 and 150 ppm for Vismodegib. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 13092 + 8009 x ($r^2$= 0.999). Linearity values can shown in Table: 2

| LEVEL | CONCENTRATION of Vismodegib in PPM | Peak Area |
|---|---|---|
| Level 1 | 0 | 0 |
| Level 2 | 25 | 186432 |
| Level 3 | 50 | 386421 |
| Level 4 | 75 | 564057 |
| Level 5 | 100 | 786843 |
| Level 6 | 125 | 989765 |
| Level 7 | 150 | 1199879 |
| Range 25 ppm to 150 ppm | Slope<br>Intercept<br>Correlation coefficient | 8009.607<br>13092.4<br>0.999615 |

**Table.2 Linearity**




**Graph.1**

**Precision:**

To study precision, six replicate standard solutions of Vismodegib 75 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (%

RSD) for peak responses was calculated and it was found to be well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

**Precision Results for Vismodegib:**

| Sample | Conc. (in ppm) | Injection No. | Peak Areas | Intra day RSD (Acceptance criteria ≤ 2.0%) |
|---|---|---|---|---|
| Vismodegib | 75 | 1 | 563942 | 0.506 |
| | | 2 | 565864 | |
| | | 3 | 569832 | |
| | | 4 | 562102 | |
| | | 5 | 567463 | |
| | | 6 | 563422 | |

**Table.3**

| Sample | Conc. (in ppm) | Injection No. | Peak Areas | Inter day RSD (Acceptance criteria ≤ 2.0%) |
|---|---|---|---|---|
| Vismodegib | 75 | 1 | 569784 | 0.1385 |
| | | 2 | 570125 | |
| | | 3 | 568975 | |
| | | 4 | 570241 | |
| | | 5 | 571021 | |
| | | 6 | 571076 | |

**Table.4**

**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 ppm dilution Peak was not clearly observed, based on which 2.5ppm is considered as Limit of Detection and Limit of Quantification is 8.25 ppm.

| Parameter | Measured Value |
|---|---|
| Limit of Quantification | 8.25 ppm |
| Limit of Detection | 2.5 ppm |

**Ruggedness:**

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days. Ruggedness also expressed in terms of percentage relative standard deviation.

| Sample (µg/ml) | Area |
|---|---|
| 1 | 568812 |
| 2 | 567901 |
| 3 | 568903 |
| 4 | 569098 |
| 5 | 563427 |
| 6 | 568120 |
| **RSD** | 0.3787 |

**Table.5**

**Robustness**

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test described above.

| S.NO | Parameter | Change | Area | % of Change |
|---|---|---|---|---|
| 1 | Standard | ..... | 564057 | ..... |
| 2 | Mobile Phase | MeOH:ACN:H2O<br>65:15:20<br>45:35:20 | <br>564987<br>569743 | <br>0.16<br>1.008 |
| 3 | $P^H$ | 5.9<br>6.3 | 570124<br>564983 | 1.07<br>0.16 |
| 4 | Wave length | 229nm<br>225nm | 570231<br>567680 | 1.09<br>064 |

**Table.6**

**Recovery**

Recovery test was performed at 3 different concentrations i.e. 75ppm, 100ppm, 125ppm. Results are given in table.7

| % Recovery | Target Conc., (ppm) | Spiked conc., (ppm) | Final Conc. (ppm) | 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 | 10158 |

**Table.7**

**Formulation Analysis:**

| S.NO | Tablet | Dosage | Sample conc. | Sample estimated | % of Drug Estimated in Tablet |
|---|---|---|---|---|---|
| 1 | Eriredge | 50 mg | 75 ppm | 74.505 | 99.34 |

**Table.8: Formulation results**

**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. Although the method could effectively separate the drug from its products,

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