Journal of
Chromatography and Spectroscopy Techniques

J Chromatogr Spectrosc Tech. 2023;1(1):11-16.
https://doi.org/10.5281/jcst.11205423

Research Article

Development and Validation of a Novel RP-HPLC Method for Estimating Isomeric

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Received: March 28, 2023; **Accepted:** April 21, 2023; **Published:** April 28, 2023

Citation: Pulusu VS, Kommarajula P. Development and Validation of a Novel RP-HPLC Method for Estimating Isomeric. J Chromatogr Spectrosc Tech. 2023;1(1):11-16.

ABSTRACT

An analytical method using high performance liquid chromatography equipped with photodiode array detector (HPLC-PDA) was developed and validated for estimation of Vismodegib as per ICH and FDA guidelines. Mobile phase consisted 0.1% Orthophosphoric acid: Acetonitrile (50:50, v/v) pumped at flow rate of 1.0 mL /min, in isocratic mode and detector was set at 264 nm. The method was validated in terms of the precision, linearity, accuracy, degradation and robustness. The detector response of Vismodegib is directly proportional to concentration ranging from 0.012-0.120 mg/mL and the mean recovery was 99.6% (RSD=0.19%, n=9). In the intra-and inter assay, the percentage difference was found to be less than 2%. Robustness was proved performing variation in mobile phase, flow rate and column temperature. A forced degradation study of Vismodegib was conducted under the condition of acidic, basic, thermal, peroxide, photo and hydrolysis. Vismodegib was found to be degraded (water bath at 60°C for 2 hours) in peroxide stress. The result of the study showed that the proposed method is simple, fast, precise and accurate, which is useful for the routine determination of Vismodegib in bulk and dosage forms.

Keywords: Vismodegib; Method development; RP-HPLC; Validation; Forced degradation.

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 [1]. Vismodegib is a crystalline free base with a pKa (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) [2]. Vismodegib is chemically known as 2-Chloro-N-(4-chloro-3-(pyridine-2-yl)phenyl)-4-(methylsulfonyl) benzamide and it's molecular formula $C_{19}H_{14}Cl_2N_2O_3S$ (Figure 1). The mechanism of action of Vismodegib binds to and inhibits smoothened, a transmembrane protein involved in Hedgehog signal transduction [3]. 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.

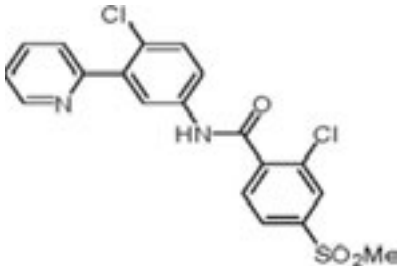

Figure 1: Chemical structure of Vismodegib.

MATERIALS AND METHODS

Chemicals and Reagents

Vismodegib standard (Purity ≥ 99.7 as is basis), Acetonitrile (HPLC grade), HPLC grade water (Millipore), 85% Orthophosphoric acid, Sodium hydroxide, Hydrochloric acid and Hydrogen peroxide were purchased from Merck.

Instrumentation

The instrument used in the study were electronic balance (Make: Mettler Toledo, Model: XP56), sonicator (Make: Elma, Model: S300H), hot air oven (Make: Serve well Instruments, Model: H02436), digital pH meter (Make: Mettler Toledo) and UV-Visible chamber (Make: Mack Equipment, Model: MK-2). HPLC (Waters,2695 with PDA detector 2996) was monitored and integrated using empower software.

Chromatographic Conditions

Chromatographic analysis was performed on the isocratic mode with a mobile phase consisting of 0.1% phosphoric acid and Acetonitrile in the proportion of 50:50 (v/v). Prior to use the mobile phase was filtered through 0.45 µm membrane filter and degassed for 10 min. The analysis was carried out on Waters 2695 series HPLC system with empower software. The analytes were conducted on an analytical column C18, (150 × 4.6) mm, 5 µm Zodiac, with a detection wavelength of 264 nm. The operating temperature of the column was 30°C and run time was 6 minutes. The injection volume was 10 µL, and the flow rate was maintained at 1.0 mL/min.

Method Development

Selection and preparation of mobile phase: In order to select a suitable mobile phase for the analysis of Vismodegib, various ratios of buffers and organic solvents were tried on the basis of pKa and some trials. The most suitable mobile phase was found to be 0.1%.

Orthophosphoric acid: Acetonitrile in the ratio of 50:50 while considering the system suitability parameters such as retention time, tailing factor, number of theoretical plates. The mobile phase was filtered through 0.45 µm membrane filter and degassed by sonication. The optimization of mobile phase buffers was captured in Table 1.

Standard preparation: The standard solution of Vismodegib prepared as 30 mg of standard was dissolved in 50 mL volumetric flask and diluted to volume with mobile phase (Stock concentration 0.6 mg/mL). Further 5.0 mL of stock was diluted to 50 mL with mobile phase and mixed. (working std. concentration 0.06 mg/mL). The representative chromatogram of Vismodegib and peak purity were shown in Figure 2 for the proposed method.

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" [4]. 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" [5]. In precision analysis, system precision, method precision and intermediate precision have been carried out.

Robustness

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 are recommended [4]. From the standard stock solution, a series of solution were prepared at a concentration levels ranging 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 verses concentration data

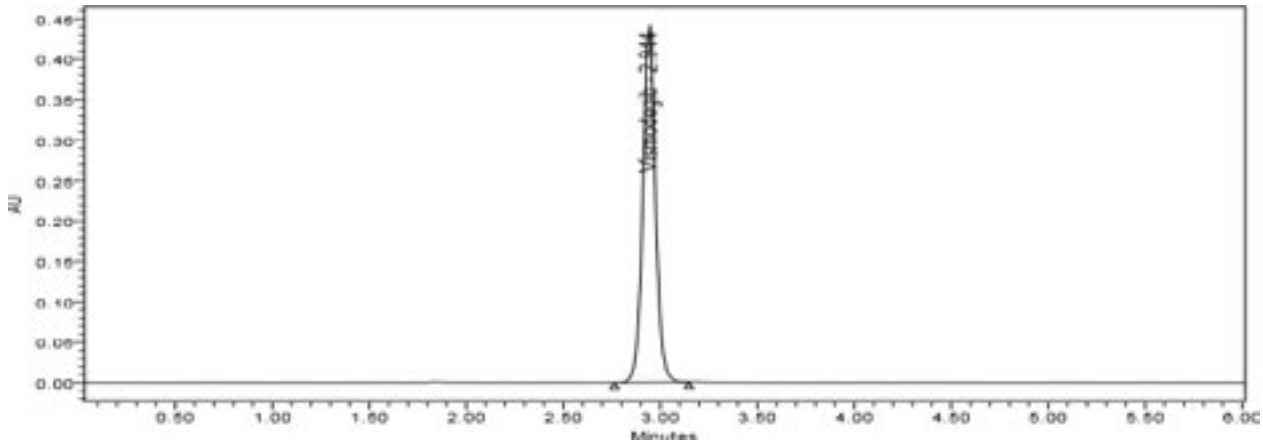

Figure 2: Typical chromatogram of Vismodegib under optimised chromatographic conditions.

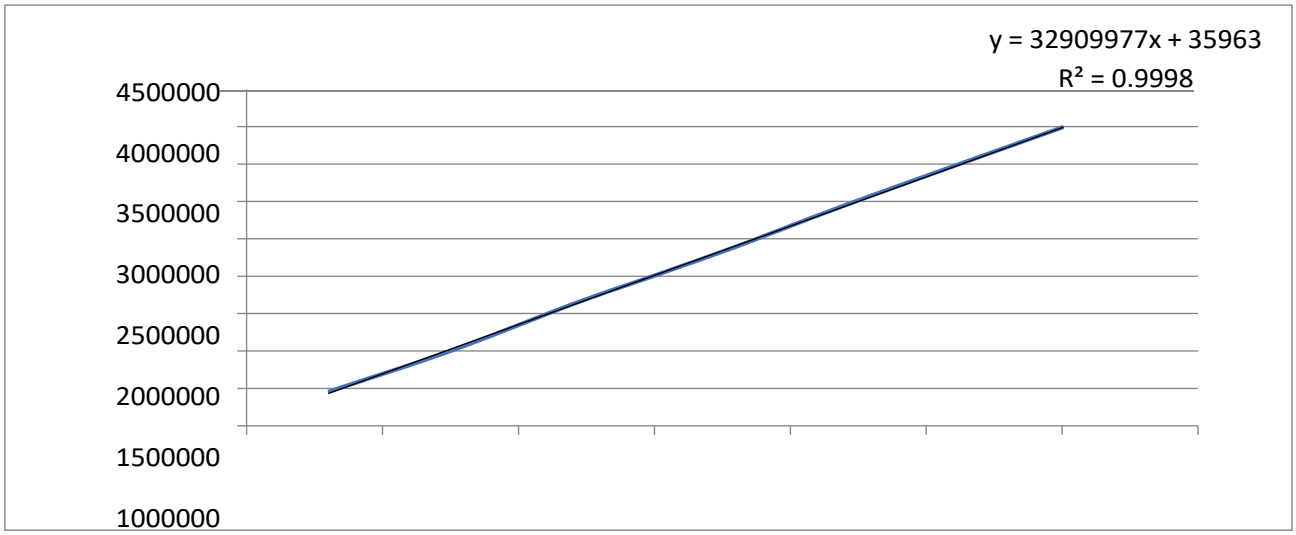

Figure 3: Linearity curve of Vismodegib.

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 [6]. 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:

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

Robustness

Robustness of the method was investigated by varying the instrumental conditions such as flow rate (± 0.2 mL/min), mobile phase composition (± 10% absolute) and column temperature (± 5°C). System suitability criteria of the standard solution was 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

S. No	Mobile phase	Remarks
Trial-1	0.1% Orthophosphoric Acid: ACN	Symmetrical peak shape
Trial-2	10 mM $Na_2HPO_4.H_2O$ (pH 4.9): ACN	Good peak shape but salt used
Trial-3	10 mM $Na_2HPO_4.H_2O$ (pH 4.9): Methanol	Late elution
Trial-4	10 mM KH_2PO_4: Methanol	Broad peak and late elution
Trial-5	10 mM KH_2PO_4: Acetonitrile	Good peak shape but salt used
Trial-6	0.1% Orthophosphoric Acid: Methanol	Broad peak and late elution

Table 1: Optimization of mobile phase and impact on chromatography.

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 [7]. Therefore, it is necessary to control the degradation to a desired level [7].

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°C increment above that for routine accelerated testing, and humidity at 75% relative humidity or greater [8]. 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°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.

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.

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 degradants [7]. The type and extent of degradation depends on the functional groups of the drug molecule and the stress conditions [7].

Peroxide degradation of Vismodegib was performed using 3% Hydrogen peroxide. Added 5.0 mL of 3% H2O2 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.

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 [7]. 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 [7]. The presence of

the C=C, C=O, Aryl chloride, $C_6H_4Cl_2$, Nitroaromatic group, $-C_6H_4NO_2$, a weak C-H bond, Sulphides, alkanes, polyenes, and phenols chemical function groups in the drug molecules is usually necessary for the occurrence of photochemical reactions [9].

Vismodegib drug substance was transferred into a quartz container and exposed to light about 1.2×10^6 lux-hours. The exposed drug was diluted as per required concentration with sample solvent and analyzed.

Hydrolysis Degradation

Hydrolysis degradation of Vismodegib drug substance was performed using distilled water. Added 5.0 mL of distilled water and kept in water bath at 60°C for 1 hour. After attained room temperature, Sample was diluted as per required concentration with sample solvent and mixed (Figure 3).

RESULTS

Method Development

Chromatographic separation: Method development was performed by screening organic modifiers (Acetonitrile and Methanol) and different strengths of buffers (Phosphate and Phosphoric acid). The final optimized mobile phase composition was found to be 0.1% phosphoric acid and Acetonitrile in the proportion of 50:50 (v/v). and run time was 6 min. The analysis was conducted at 264 nm with Zodiac C18 column (150 × 4.6) mm, 5 µm particle size and the column temperature was 30°C. The injection volume was 10 µL and the isocratic mobile phase flow rate was 1.0 mL/min.

Method Validation

Linearity: The standard stock was used for linearity stock, serial dilutions were prepared and covered the range from 0.012 mg/mL to 0.120 mg/mL. Three replicate injections of each concentration were analyzed for this study and regression coefficient (r2) was found to be 0.9998.

Precision: The HPLC is found to be precise at the % RSD of the area counts for six standard injections was 0.89. The method precision and intermediate precision samples were prepared at 100% of the sample target concentration and evaluated six samples as per method. The peak area was measured and % RSD was calculated. The developed method also precise as the % RSD of amount present for Vismodegib in the method precision sample was 0.22 and intermediate precision sample was 0.12.

Accuracy: The accuracy of the method was determined by analyzing samples at three different (50%, 100% and 150%) levels of concentrations by spiking Vismodegib active substance. The % recovery is found within the acceptable criteria, also individual and overall % RSD of recovery is found within the acceptable criteria; hence it shows that the method is accurate.

Forced degradation studies: These stress conditions produce minute or no degradation due to the nature of a drug molecule. The nature of degradation depends on the functional groups of the drug molecule and the effect of stress conditions.

Robustness: Robustness of the method was studied by deliberate variation of the analytical parameters such as flow (1.0 ± 0.2mL/min), column temperature (30 ± 5°C) and mobile phase composition (± 10% absolute).

DISCUSSION

No stability indicating method is available in the official compendia using HPLC for analyzing Vismodegib in bulk and formulation dosage forms until now. The present work has been reported with an intention to develop a new validated method for the estimation of Vismodegib by RP-HPLC method using a photo diode array detector. Different makes of C18 columns, buffers and organic solvents were used to develop a stability indicating method. To achieve symmetric peak shape, mobile phase prepared with 0.1% orthophosphoric acid (pH 2.50) and acetonitrile in the ratio of 1:1, column was selected Zodiac C18, (150 × 4.6) mm, 5 µm at a flow rate of 1.0 mL/min and column temperature at 30°C. Under the above optimized conditions, the retention time of Vismodegib was 3.0 min. The linearity of an analytical procedure was demonstrated by preparing at seven different concentrations from 0.012 mg/mL to 0.120 mg/mL and analyzed.

The calibration curve constructed for Vismodegib by plotting the peak area versus concentration and the regression coefficient (R2) is 0.9998. The mean recovery was 99.6 and this molecule was sensitive to peroxide degradation. The degradation was achieved around 6.5% with 3% Hydrogen peroxide at 60°C for 1 hour. Robustness study was performed with variation of flow, mobile phase composition and column temperature but none of the above variations resulted in critical changes to the system analysis parameters. This method was developed and validated for precision, Linearity, accuracy, degradation and robustness as per ICH and FDA guidelines. Hence, the purpose of developing an accurate and stable method along with its validation was achieved for Vismodegib.

CONCLUSION

A robust and accurate RP-HPLC assay method was developed and validated for estimation of Vismodegib as per ICH/

FDA guidelines. This method was validated by using various validation parameters such as system suitability, method and intermediate precision, linearity, accuracy, degradation and robustness.

Degradation was performed under acidic, basic, thermal, photolytic, peroxide and hydrolysis conditions. The developed method was sensitive, precise and capable of determine the impurities in stress study and interference as well. Apart from these, this is low cost analytical methodology, shorter run time and it can be applied in various laboratories for estimation of Vismodegib in bulk and pharmaceutical dosage form.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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