Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2025.v13.i04.A10

DEVELOPMENT AND OPTIMIZATION OF A VALIDATED RP-HPLC METHOD FOR THE QUANTIFICATION OF VISMODEGIB IN PHARMACEUTICAL DOSAGE FORMS

V. M. Nishad[*1] and Manoj Dilip Patil[2]

[1]*School of Pharmacy, Sunrise University, Alwar, Rajasthan, India.

[2]Shinde Institute of Pharmacy and Research, Pachora, Jalgaon, Maharashtra, India.

ABSTRACT

A simple, rapid and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Vismodegib in pharmaceutical capsule dosage forms. Chromatographic separation was achieved on an Inertsil C18 column (150 × 4.6mm, 5µm) using a mobile phase consisting of 0.01 N ammonium phosphate buffer and acetonitrile in the ratio of 55:45 (v/v), delivered at a flow rate of 0.9mL/min. Detection was carried out at 258nm with a total run time of 6 minutes. The method showed a retention time of approximately 2.56 minutes for Vismodegib. Validation was performed in accordance with ICH Q2(R1) guidelines. The method demonstrated excellent linearity over the concentration range of 7.5-45µg/mL with a correlation coefficient (R2) of 0.999. Precision and accuracy studies showed %RSD values below 2% and recovery between 98.0% and 102.0%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.16µg/mL and 0.48µg/mL, respectively, indicating good sensitivity. Robustness studies confirmed that minor variations in chromatographic conditions did not significantly affect the results. Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic and neutral conditions demonstrated that the method is stability-indicating, with effective separation of degradation products from the analyte peak. The validated method was successfully applied to the assay of marketed Vismodegib capsules, yielding an assay value of 99.66%. The proposed RP-HPLC method is simple, precise, accurate, robust and suitable for routine quality control and stability analysis of Vismodegib in pharmaceutical formulations.

KEYWORDS

Vismodegib, Pharmaceutical dosage forms and RP-HPLC method.

Author for Correspondence:
Nishad V M,
School of Pharmacy,
Sunrise University, Alwar, Rajasthan, India.
Email: nishadvm13@gmail.com

INTRODUCTION

Vismodegib is an orally active Hedgehog pathway inhibitor approved for the treatment of locally advanced and metastatic basal cell carcinoma (Sekulic *et al*, 2012[1], U.S. Food and Drug Administration [FDA], 2012)[2]. The Hedgehog

signaling pathway plays a crucial role in embryonic development, tissue regeneration, and cellular differentiation and its aberrant activation has been implicated in the pathogenesis of various malignancies (Rubin and De Sauvage, 2006)[3]. Vismodegib exerts its pharmacological action by selectively inhibiting the Smoothened (SMO) receptor, thereby suppressing abnormal Hedgehog pathway signaling and tumor progression.

Given its clinical importance and narrow therapeutic margin, stringent quality control of pharmaceutical formulations containing Vismodegib is essential to ensure safety, efficacy and batch-to-batch consistency. Accurate and reliable analytical methods are therefore required during drug development, manufacturing and post-marketing surveillance. Several analytical techniques, including liquid chromatography-tandem mass spectrometry (LC-MS/MS), have been reported for the determination of Vismodegib in biological matrices (Al Shirity *et al*, 2023)[4]. However, such techniques involve high operational costs, require specialized instrumentation, and are not routinely available in many quality control laboratories.

High-performance liquid chromatography (HPLC) remains one of the most widely used and accepted analytical techniques in pharmaceutical analysis due to its high resolution, reproducibility, sensitivity, and versatility (Snyder *et al*, 2010)[5]. HPLC plays a central role in the qualitative and quantitative analysis of active pharmaceutical ingredients, impurities, and degradation products, making it indispensable for routine quality control and regulatory compliance (Swartz and Krull, 2012)[2]. Reverse-phase HPLC (RP-HPLC), in particular, is preferred for the analysis of moderately lipophilic compounds such as Vismodegib because of its robustness, simplicity and compatibility with UV detection systems.

Furthermore, regulatory agencies emphasize the development of stability-indicating analytical methods to assess drug stability and ensure product quality throughout its shelf life (International Council for Harmonisation [ICH], 2005)[6]. A stability-indicating RP-HPLC method is capable of effectively separating the drug substance from its degradation products under various stress conditions, thereby ensuring method specificity and reliability.

In this context, the objective of the present study was to develop and validate a simple, accurate, precise and stability-indicating RP-HPLC method for the estimation of Vismodegib in capsule dosage forms, in accordance with ICH Q2(R1) guidelines. The proposed method aims to provide a cost-effective and reliable analytical tool suitable for routine quality control and stability testing in pharmaceutical laboratories.

MATERIAL AND METHODS

Chemicals and Reagents

Vismodegib reference standard was obtained from a certified supplier. Dibasic ammonium phosphate, acetonitrile (HPLC grade), and Milli-Q water were used throughout the study. All reagents and solvents used were of analytical or HPLC grade, as recommended for chromatographic method development (Snyder *et al*, 2010)[5].

Instrumentation

The chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with a UV detector. Data acquisition and processing were carried out using standard chromatography software. Separation was achieved on an Inertsil C18 column (150 × 4.6mm, 5µm), which is commonly employed for the analysis of moderately lipophilic pharmaceutical compounds (Snyder *et al*, 2010)[5].

Chromatographic Conditions

The chromatographic separation was carried out under the following optimized conditions:

Mobile phase: 0.01N ammonium phosphate buffer: acetonitrile (55:45 v/v)
Flow rate: 0.9mL/min
Detection wavelength: 258 nm
Column temperature: 26°C
Injection volume: 10µL
Run time: 6.0 minutes

These conditions were selected to achieve optimal resolution, peak symmetry, and sensitivity.

Preparation of Standard and Sample Solutions

A standard stock solution was prepared by accurately weighing 15mg of Vismodegib and dissolving it in 50 mL of diluent to obtain a concentration of 300µg/mL. Appropriate dilutions of the stock solution were made with the same diluent to obtain working standard solutions within the linearity range.

The sample solution was prepared by accurately weighing capsule powder equivalent to 150mg of Vismodegib, followed by dilution with diluent, sonication to ensure complete extraction of the drug, filtration through a suitable membrane filter, and further dilution to obtain a final concentration of 30µg/mL. The sample preparation procedure was designed to minimize matrix interference and ensure reproducible results.

Method Validation

The method was validated in accordance with ICH Q2(R1) guidelines.

RESULTS AND DISCUSSION

Method Optimization

Optimization of chromatographic conditions was carried out to achieve a sharp, symmetrical peak with good resolution and minimal run time, as recommended for RP-HPLC method development (Snyder *et al*, 2010)[5]. Different combinations of buffer concentration, organic solvent ratio, and flow rate were examined to improve peak shape and sensitivity. The optimal separation of Vismodegib was obtained using a mobile phase composed of 0.01N ammonium phosphate buffer and acetonitrile in the ratio of 55:45 (v/v), delivered at a flow rate of 0.9mL/min. Detection at 258nm resulted in adequate sensitivity with minimal baseline interference, consistent with UV-based HPLC methods reported for pharmaceutical compounds (Blessy *et al*, 2014)[7]. Under these optimized conditions, Vismodegib eluted at a retention time of approximately 2.56 minutes.

System Suitability

System suitability parameters were evaluated by six replicate injections of the standard solution in accordance with ICH guidelines (ICH Q2(R1), 2005). The results demonstrated consistent retention time, peak area, and peak symmetry. The number of theoretical plates exceeded 8600, indicating good column efficiency, and the tailing factor was approximately 1.35. The %RSD of peak areas was found to be less than 2%, confirming acceptable system precision for routine analytical applications.

Linearity

Linearity of the method was evaluated over the concentration range of 7.5-45µg/mL as per validation requirements outlined in ICH guidelines (ICH Q2(R1), 2005). A calibration curve constructed by plotting peak area versus concentration showed a linear response. The regression equation was $y = 116623x + 27789$, with a correlation coefficient ($R^2 = 0.999$), demonstrating excellent linearity across the studied range.

Precision

Method precision was assessed through repeatability studies by analyzing six replicate injections of the sample solution at the working concentration. The %RSD of peak areas was below 2%, indicating good repeatability and reliability of the method. These results comply with accepted precision limits for analytical methods used in pharmaceutical quality control (ICH Q2(R1), 2005).

Accuracy

Accuracy was determined by recovery studies using the standard addition method at 50%, 100%, and 150% levels, following standard analytical validation practices (Blessy *et al*, 2014). The percentage recovery of Vismodegib ranged from 98.0% to 102.0%, with low %RSD values, confirming the accuracy of the method and the absence of interference from formulation excipients.

Robustness

Robustness of the method was evaluated by introducing deliberate small changes in chromatographic conditions, including flow rate (±0.1mL/min), mobile phase composition (±5%), and column temperature (±2°C), as recommended by regulatory guidelines (ICH Q2(R1), 2005). These variations did not significantly affect retention time, peak area, or system suitability parameters. The %RSD values remained below 2%, indicating that the method is robust.

Sensitivity

The sensitivity of the method was assessed by determining the limit of detection (LOD) and limit of quantification (LOQ) using signal-to-noise ratios as described in ICH guidelines (ICH Q2(R1), 2005). The LOD and LOQ were found to be 0.16µg/mL and 0.48µg/mL, respectively, demonstrating that the method is sufficiently sensitive for routine quantitative analysis.

Forced Degradation Studies

Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, photolytic, and neutral conditions to evaluate the stability-indicating nature of the method (Blessy *et al*, 2014)[7]. Vismodegib exhibited degradation under stress conditions; however, the degradation products were well resolved from the main analyte peak. No interference was observed at the retention time of Vismodegib, confirming the specificity and stability-indicating capability of the method.

Assay of Marketed Formulation

The validated method was applied to the assay of commercially available Vismodegib capsules (ERIVEDGE®). The mean assay value was found to be 99.66% of the labeled claim, with a %RSD of 0.47, demonstrating the suitability of the method for routine quality control analysis in accordance with pharmacopeial expectations.

Discussion

The development of a reliable analytical method for Vismodegib is essential to ensure the quality, safety, and consistency of pharmaceutical formulations, particularly for anticancer drugs requiring strict quality control (Sekulic *et al*, 2012)[1]. In the present study, a simple and efficient RP-HPLC method was successfully developed and validated in accordance with ICH Q2(R1) guidelines.

The optimized chromatographic conditions resulted in a short retention time and good peak symmetry, enabling rapid analysis without compromising resolution. The use of a buffered mobile phase contributed to stable retention behavior and improved peak shape, while acetonitrile provided adequate elution strength, which is consistent with established RP-HPLC method development principles (Snyder *et al*, 2010)[5].

System suitability results demonstrated the reliability and reproducibility of the chromatographic system. The high number of theoretical plates and acceptable tailing factor reflected efficient column performance. The excellent linearity observed over the studied concentration range confirmed the suitability of the method for quantitative estimation of Vismodegib in pharmaceutical dosage forms (ICH Q2(R1), 2005)[6].

Precision and accuracy studies showed low variability and high recovery values, indicating that the method produces consistent and accurate results. Robustness testing confirmed that minor variations in analytical conditions do not significantly affect method performance, which is essential for routine quality control environments.

The low LOD and LOQ values demonstrated the sensitivity of the method, making it suitable for detecting and quantifying low levels of Vismodegib. Furthermore, forced degradation studies established that the method is stability-indicating, as degradation products were clearly separated from the analyte peak, ensuring specificity during stability testing (Blessy *et al*, 2014)[7].

The successful application of the method to the assay of marketed Vismodegib capsules further confirmed its practical utility. Overall, the developed RP-HPLC method is simple, rapid, precise, accurate, and stability-indicating, and can be effectively employed for routine quality control and stability assessment of Vismodegib in pharmaceutical dosage forms.

CONCLUSION

A simple, rapid, accurate and stability-indicating RP-HPLC method was successfully developed and validated for the quantitative estimation of Vismodegib in pharmaceutical capsule dosage forms. The method was validated in accordance with ICH Q2(R1) guidelines and demonstrated excellent linearity, precision, accuracy, robustness, and sensitivity. The optimized chromatographic

conditions resulted in a short retention time with good peak symmetry, enabling efficient analysis suitable for routine laboratory use.

Forced degradation studies confirmed that the method is stability-indicating, as degradation products were well separated from the analyte peak without interference. The validated method was successfully applied to the assay of a marketed Vismodegib formulation, demonstrating its practical applicability for routine quality control and stability testing.

Overall, the proposed RP-HPLC method is cost-effective, reliable and reproducible, making it suitable for routine analysis of Vismodegib in pharmaceutical formulations in quality control and regulatory laboratories.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to School of Pharmacy, Sunrise University, Alwar, Rajasthan, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Sekulic A, Migden M R, Oro A E, Dirix L, Lewis K D, Hainsworth J D, Hauschild A. Efficacy and safety of vismodegib in advanced basal-cell carcinoma, *New England Journal of Medicine,* 366(23), 2012, 2171-2179.
2. Swartz M E, Krull I S. *Analytical method development and validation*, *CRC Press, U.S. Food and Drug Administration (FDA),* 2012.
3. Rubin L L, De Sauvage F J. Targeting the Hedgehog pathway in cancer, *Nature Reviews Drug Discovery*, 5(12), 2006, 1026-1033.
4. Al Shirity Z N, Westra N, Van Hateren K, Oude Munnink T H, Kosterink J G W, Mian P, Lub-de Hooge M N, Touw D J, Gareb B. Validation of an LC-MS/MS assay for rapid and simultaneous quantification of 21 kinase inhibitors in human plasma and serum for therapeutic drug monitoring, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences,* 1229, 2023, 123872.
5. Snyder L R, Kirkland J J, Dolan J W. *Introduction to modern liquid chromatography, Hoboken, NJ: John Wiley and Sons,* 3rd Edition, 2010.
6. International Council for Harmonisation (ICH), *Validation of analytical procedures: Text and methodology Q2(R1), ICH Harmonised Tripartite Guideline,* 2005.
7. Blessy M, Patel R D, Prajapati P N, Agrawal Y K. Development of forced degradation and stability indicating studies of drugs-A review, *Journal of Pharmaceutical Analysis,* 4(3), 2014, 159-165.

Please cite this article in press as: Nishad V M and Manoj Dilip Patil. Development and optimization of a validated RP-HPLC method for the quantification of Vismodegib in pharmaceutical dosage forms, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 13(4), 2025, 64-68.