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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF VIMSELTINIB BY USING HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Vimseltinib in bulk and pharmaceutical dosage form. Chromatographic separation of Vimseltinib was achieved on Waters Alliance-e2695, by using Waters Agilent Eclipse XDB (150x4.6 mm, 3.5µm) column and the mobile phase containing Acetonitrile and 0.1% Formic acid in the ratio of 20:80% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 221nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Vimseltinib were NLT 2000 and should not more than 2, respectively. % Relative standard deviation of peak areas of all measurements is always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be a simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Vimseltinib.

Keywords: *HPLC, Vimseltinib,Formic acid,Acetonitrile,ICH guidelines.*

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INTRODUCTION

Vimseltinib (molecular formula $C_{23}H_{25}N_7O_2$, molecular weight 431.5 g/mol) is an orally active, small-molecule switch-control tyrosine kinase inhibitor developed as a selective and potent antagonist of colony-stimulating factor 1 receptor (CSF1R). It is described as a lipophilic compound with low aqueous solubility but good permeability, typically soluble in organic solvents such as DMSO, consistent with the physicochemical behaviour of many kinase inhibitors [1]. Therapeutically, Vimseltinib is classified as an antineoplastic agent indicated for adult patients with symptomatic tenosynovial giant cell tumour (TGCT) when surgical intervention is not feasible or may worsen functional outcomes. Mechanistically, it stabilises CSF1R in its inactive conformation via the switch-control region, thereby preventing receptor autophosphorylation, ligand-induced downstream signalling, and proliferation of CSF1R-dependent cells. Pharmacokinetic studies show rapid absorption with a median Tmax of approximately 1 hour, dose-dependent increases in Cmax and AUC, and no clinically significant food effect. Clinically relevant adverse effects primarily include hepatotoxicity, warranting monitoring of liver function, alongside standard precautions regarding reproductive safety [2].Till today, there is no Literature review on the method development and validation of Vimseltinib.

MATERIALS AND METHODOLOGY

Reagents & Chemicals

Vimseltinibis purchased from Simson Pharma Limited. Analytical reagent (AR) grade formic acid and ortho-phosphoric acid (Merck) were utilised for pH adjustment and buffer preparation.

Instrumentation

The chromatographic analysis was performed using a Waters Alliance HPLC system equipped with an isocratic pump. UV/VIS measurements were obtained with a Shimadzu UV-1700 spectrophotometer, and all volumetric glassware used was Class-A grade (Borosil).

Preparation of standard solution

Accurately weigh and transfer 9mg of Vimseltinib working standard into a 10 ml volumetric flask, add Diluent, and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. (Stock solution).Further pipette 1ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (90ppm of Vimseltinib)

Sample Solution Preparation

Accurately weigh and transfer 27.3mg of Vimseltinib sample into a 10mL volumetric flask, add Diluent and sonicate it for up to 30 min to dissolve it completely and make the volume up to the mark with the same solvent. (Stock solution).Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Then it is filtered through a 0.45 micron Injection filter. (90ppm of Vimseltinib)

Determination of Working Wavelength (λ_{max})

In the estimation of drug maximum absorbance was used. So, this wavelength was used to accurately estimate the Vimseltinib drug. The wavelength of maximum absorption of the solution of the drug in a mixture of Acetonitrile and 0.1% Formic acid (20:80) was scanned using a PDA Detector within the wavelength region of 200–400 nm against[3].

Method Validation

System Suitability

Six duplicate injections containing Vimseltinib at 90 µg/mL were produced to ascertain System Suitability [4].

Specificity

To assess specificity, an analytical method must be capable of identifying a target analyte exclusively among controls and known impurities. To accomplish this, chromatograms were acquired for standards, blanks, and samples [5].

Linearity and LOD, LOQ

The developed method's linearity was evaluated by injecting 22-135 µg/mL of Vimseltinib. Triplets are produced and administered into the chromatographic system. The linearity graph plots the peak area against concentration, where concentration- X- axis and Peak area -Y-axis are represented in µg/mL.

The minimum number of analytes that were identifiable, nevertheless not quantified in a sample, is the lowest analyte number that an analytical technique can detect but not necessarily quantify. This is the point at which the analyte or compound's instrument response can be separated from the instrument noise. LOQ was a specification in analytical chemistry that specifies the lowest possible concentration of analytes which are measurable with adequate accuracy and precision [6-8].

Accuracy

This method's accuracy was evaluated using the % recovery method. Solutions at 80, 100 and 120 % concentration levels were prepared, with three replicates of each concentration [9,10].

Precision

Method precision indicates the precision observed over identical experimental conditions (same instrument in the same lab on the same day) over a short period. The study of precision under varied experimental conditions, like different equipment, labs, and days, was termed intermediate precision. Precision was expressed as % RSD and should be < 2 per ICH guidelines [11,12].

Robustness

To assess the robustness of a method, variations in specifications, for instance, such as the % of organic solvent, pH value, ionic strength, and temperature, are applied. The changing components that make up robustness research are usually parameters that the technique states. Variability in the resultant data is noted in the approach procedure so that the analyst may take steps to control these variables closely. This would ensure that the analysis yielded consistent findings each time[13,14].

Assay

The HPLC system was injected with standard and sample solutions containingVimseltinib. The % purity was computed using the peak area response values obtained [15].

Degradation Studies

Forced degradation studies were conducted to confirm the stability-indicating nature of the HPLC method for vimseltinib. Samples (27.3 mg) were subjected to acid (1 N HCl), alkali (1 N NaOH), oxidative (10% w/v H_2O_2), reductive (10% w/v sodium bisulphite), hydrolytic (water), photolytic (3 h exposure), and thermal (105°C, 3 h) stress conditions. Acidic and alkaline samples were neutralized after heating at 60°C for 1 hour. All stressed solutions were diluted with diluent, filtered through a 0.45 µm syringe filter, and analyzed by HPLC [16,17].

Acceptance Criteria: Purity threshold > purity angle.

RESULTS AND DISCUSSION

Determination of Working Wavelength (λ_{max})

The wavelength of maximum absorption of the solution of the drug in a mixture of Acetonitrile and 0.1% Formic acid (20:80) was scanned using a PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% Formic acid (20:80) as a blank. Thus, 221nm was selected as the detector wavelength for the HPLC chromatographic method (Figure 1).

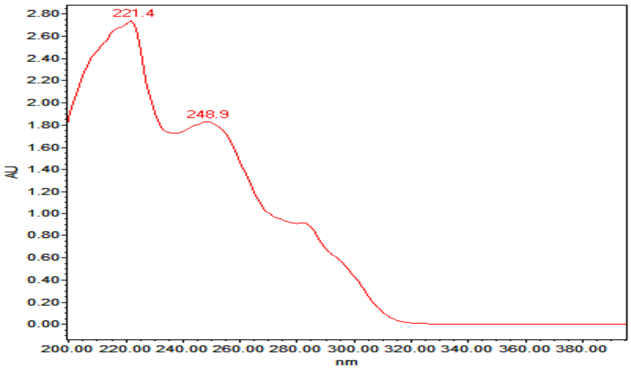


Figure 01: PDA Spectrum of Vimseltinib

Optimisation of Chromatographic Conditions

Multiple trials were performed to establish suitable chromatographic conditions for the analysis of vimseltinib. Initial experiments using a Luna C18 column with higher proportions of acetonitrile (80:20 and 70:30, acetonitrile:0.1% OPA) resulted in unacceptable retention time, plate count, and peak tailing. Further adjustment to 60:40 improved retention but produced inadequate baseline stability. Subsequent trials replacing OPA with 0.1% formic acid (35:65 and 30:70, acetonitrile: formic acid) showed suboptimal peak shape and peak height. Optimal performance was achieved using an Agilent Eclipse XDB column (150 × 4.6 mm, 3.5 μm) with a mobile phase consisting of acetonitrile and 0.1% formic acid (20:80 v/v), at a flow rate of 1.0 mL/min and detection wavelength of 221 nm. Under these conditions, vimseltinib exhibited acceptable retention, symmetric peak shape, and satisfactory system suitability parameters. Therefore, the optimised method was selected for validation (Figure 02 and Table 01).

Table 01: Optimised chromatographic conditions

Parameters	Observation
Instrument used	Waters Alliance e-2695 HPLC
Injection volume	10µl
Mobile Phase	Acetonitrile and 0.1% Formic acid (20+80)
Column	Agilent Eclipse XDB (150x4.6 mm, 3.5µm)
Detection Wave Length	221nm
Flow Rate	1 mL/min
Runtime	5min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

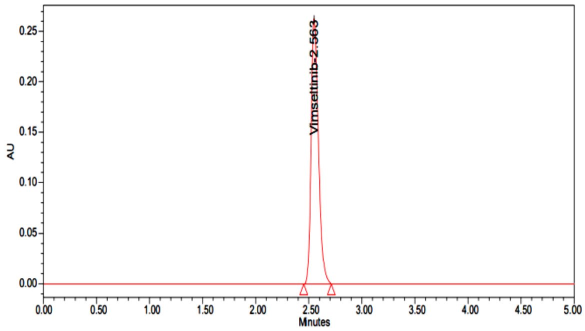


Figure 02: Optimised chromatogram of Vimseltinib

Method Validation

ICH criteria validated the optimised procedure, and the results are shown below.

System suitability

According to ICH guidelines plate count should be more than 2000, the tailing factor should be less than 2, and the resolution must be more than 2. All the system's suitable parameters were passed and were within the limits (Table 02).

Table 02: System suitability parameters for vimseltinib

S. No.	Parameter	Vimseltinib
1	Retention time (min)	2.563
2	Plate count	11203
3	Tailing factor	1.15
4	%RSD	0.48

Specificity

Retention times of Vimseltinib were 2.563 min (Figure 04). We did not found and interfering peaks in the blank and placebo at the retention times of these drugs in this method(Figure 03). So this method was said to be specific.

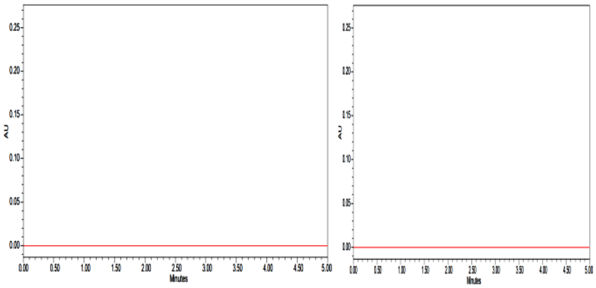


Figure 03: Chromatogram of blank and placebo

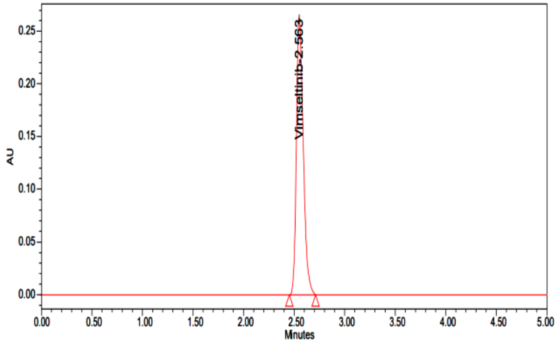


Figure 05: Spiked chromatogram

Linearity, LOD & LOQ

Linearity of vimseltinib was evaluated over the concentration range of 22.50–135.00 µg/mL. A strong linear relationship between peak area and concentration was observed, with a regression equation of y = 26546.99x + 17857.36 and a correlation coefficient (R^2) of 0.99973 (Table 3). The calibration curve (Figure 6) confirmed excellent proportionality of detector response across the studied range. These results demonstrate that the method is linear and suitable for quantitative analysis. LOD for Vimseltinib was found to be 0.54µg/mL and LOQ for Vimseltinib was found to be 1.80µg/ml.

Table 03: Results of linearity for Vimseltinib

S.NO	**Vimseltinib**	
	Conc.(µg/ml)	**Peak area**
1	22.50	608022
2	45.00	1216043
3	67.50	1824065
4	90.00	2432086
5	112.50	3040108
6	135.00	3548129
Regression equation	y = 26546.99x + 17857.36	
Slope	26546.99	
Intercept	17857.36	
R^2	0.99973	

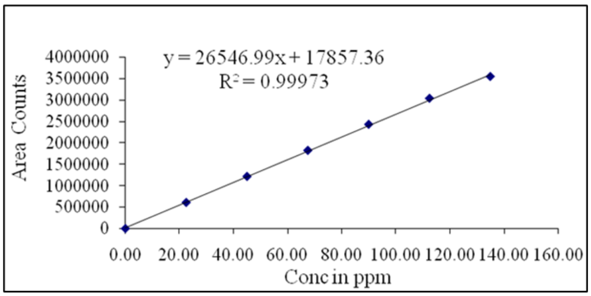


Figure 06: Calibration curve for Vimseltinib

Accuracy

Accuracy of the method was assessed at 80%, 100%, and 120% specification levels using the standard addition technique. The percentage recovery values ranged from 99.0% to 100.7%, with mean recoveries of 99.8%, 100.2%, and 99.3%, respectively (Table 4). These results confirm the accuracy and trueness of the developed HPLC method for Vimseltinib.

Table 04: Accuracy results of Vimseltinib by HPLC method

Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean %Recovery**
80%	4362870	16.2	16.228	100.2	99.8
	4318127	16.2	16.062	99.1	
	4357108	16.2	16.207	100.0	
100%	4865720	18.0	18.099	100.6	100.2
	4812637	18.0	17.901	99.5	
	4872180	18.0	18.123	100.7	
120%	5268710	19.8	19.598	99.0	99.3
	5289461	19.8	19.675	99.4	
	5298451	19.8	19.708	99.5	

Precision

System precision and method precision were evaluated by six replicate injections at 90 µg/mL. The %RSD values obtained were 0.48% for system precision and 0.71% for method precision (Table 5), both within the acceptable limit of <2%. These findings indicate excellent repeatability and precision of the developed HPLC method for vimseltinib.

Table 05: System precision results for vimseltinib

		System Precision	**Method Precision**
S. No	**VimseltinibConcentration (µg/ml)**	**Area of Vimseltinib**	**Area for Vimseltinib**
1.	90	2410369	2436187
2.	90	2421384	2423128
3.	90	2436132	2403157
4.	90	2426157	2413181
5.	90	2403120	2451308
6.	90	2420461	2416823
Mean	2419604		2423964
S. D	11636.38		17308.918
%RSD	0.48		0.71

Assay of Vimseltinib

The assay of Vimseltinib in the Romvimza formulation showed consistent chromatographic responses with minimal variation between replicate injections. The calculated amount found was 9.104 µg/mL, corresponding to a % assay of 101.2%. This value falls well within acceptable pharmacopeial limits (typically 98–102%), confirming the accuracy of the developed RP-HPLC method. The results indicate that the formulation contains the labelled drug amount without significant loss or interference.

Robustness of Vimseltinib

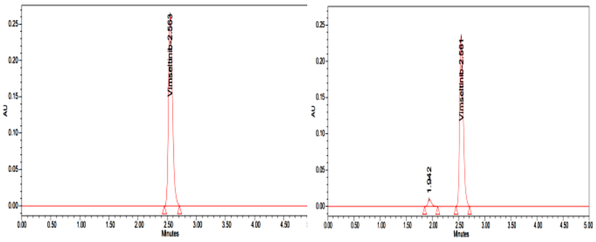
Robustness testing demonstrated that deliberate variations in flow rate and organic phase composition did not significantly affect method performance. Retention time shifted predictably with flow rate changes, while peak symmetry (tailing 1.1–1.2) and plate counts (>11,000) remained within acceptable limits. The %RSD values were below 1%, indicating excellent reproducibility. These findings confirm that the method is robust and reliable under minor operational variations.

Forced degradation studies

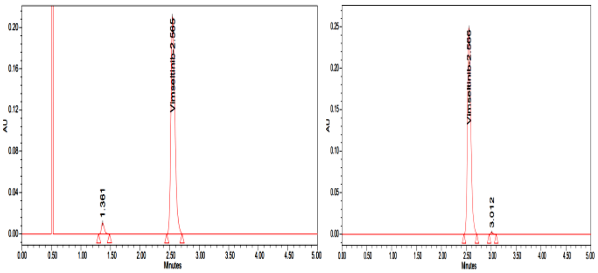
Forced degradation studies showed that Vimseltinib is most susceptible to oxidative and acidic stress, with degradations of 13.3% and 12.2%, respectively (Table 6, Figure 8). Moderate degradation was observed under reduction (8.4%), while alkali and photolytic conditions produced minimal effects (~3%). Thermal and hydrolytic stress resulted in negligible degradation (<0.5%), indicating good stability. In all cases, the purity angle remained below the purity threshold, confirming peak homogeneity and the stability-indicating nature of the method.

Table 06. Forced degradation results for vimseltinib

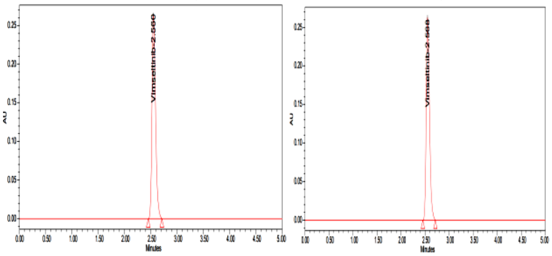
Condition	Area	% Assay	% Degradation	Purity Angle	Purity Threshold
Control	2420363	100.0	0.0	0.942	2.133
Acid	2123471	87.8	12.2	0.992	2.129
Alkali	2345168	96.9	3.1	0.901	2.176
Peroxide	2098451	86.7	13.3	0.947	2.115
Reduction	2215480	91.6	8.4	0.966	2.102
Thermal	2410328	99.6	0.4	0.985	2.151
Photolytic	2346982	97.0	3.0	0.989	2.148
Hydrolysis	2417187	99.9	0.1	0.973	2.129



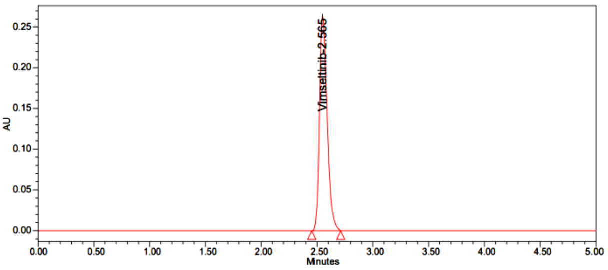
Acid degradationAlkali degradation



Peroxide degradationReduction degradation



Thermal degradationPhotolytic degradation



Chromatogram of Hydrolysis degradation

Figure 08: Chromatograms under diffrent stress conditions

CONCLUSION

The developed HPLC method for the estimation of the selected drug is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.The sample recoveries were in good agreement with their respective label claims,suggesting that formulation excipients do not interfere with estimation and that they can be used in laboratories for the routine analysis of selected drugs. The present work concluded that the stability-indicating RP-HPLC assay was simple, accurate, precise, and specific, and showed no interference from the placebo and degradation products. Hence, these can be used for routine analysis of Vimseltinib.

AUTHOR CONTRIBUTIONS

Shaileshkumar Bhingaradiya: Conceptualisation, methodology, investigation, data curation, writing – original draft. Venkata Lakshmanan Sagar Dantinapalli:

Formal analysis, validation, visualisation, writing – review & editing. Selvaraja Elumalai: Supervision, resources, project administration, writing – review & editing.

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Nil.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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