A New Analytical RP-HPLC Method for the Estimation of Vemurafenib in Pure Form and Marketed Pharmaceutical Dosage Form

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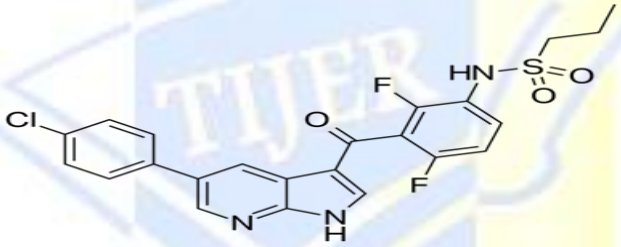
Abstract - A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Vemurafenib in bulk form and marketed formulation. Separation of Vemurafenib was successfully achieved on a Symmetry ODS C18 (4.6 x 250mm, 5µm) column in an isocratic mode of separation utilizing Acetonitrile: Methanol in the ratio of 80:20% v/v at a flow rate of 1mL/min and the detection was carried out at 272nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 10-50mcg/mL for Vemurafenib.The correlation coefficient was found to be 0.999 for Vemurafenib. The LOD and LOQ for Vemurafenib were found to be 1.1µg/mL and 3.2µg/mL respectively. The proposed method was found to be good percentage recovery for Vemurafenib, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.
Index Terms - Vemurafenib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

I.Introduction

Vemurafenib is a competitive kinase inhibitor with activity against BRAF kinase with mutations like V600E. It exerts its function by binding to the ATP-binding domain of the mutant BRAF.3 Vemurafenib was co-developed by Roche and Plexxikon and it obtained its FDA approval on August 17, 2011, under the company Hoffmann La Roche. After approval, Roche in collaboration with Genentech launched a broad development program. Vemurafenib is as elective inhibitor of BRAF kinase that is used in the therapy of patients with metastatic and advanced malignant melanoma.[1].

Fig.1 Structure of Vemurafenib



II.Literature survey

C M Nijenhuis, et al. (2023): Vemurafenib is an inhibitor of mutated serine/threonine- protein kinase B-Raf (BRAF) and is registered as Zelboraf (®) for the treatment of adult patients with BRAF V600 mutation-positive unresectable or metastatic melanoma. To support Therapeutic Drug Monitoring (TDM) and clinical trials, we developed and validated a method for the quantification of Vemurafenib in human plasma. Additionally two LC-MS systems with different detectors were tested: the TSQ Quantum Ultra and the API3000. Human plasma samples were collected in the clinic and stored at nominally - 20°C. Vemurafenib was isolated from plasma by liquid-liquid extraction, separated on a C18 column with gradient elution, and analysed with triple quadrupole mass spectrometry in positive-ion mode. A stable isotope was used as internal standard for the quantification. Ranging from 1 to 100µg/ml the assay was linear with correlation coefficients (r (2)) of 0.9985 or better. Inter-assay and intra-assay accuracies were within±7.6% of the nominal concentration; inter-assay and intra-assay precision were within≤9.3% of the nominal concentration. In addition all results were within the acceptance criteria of the US FDA and the latest EMA guidelines for method validation for both MS detectors. In conclusion, the presented analytical method for Vemurafenib in human plasma was successfully validated and the performance of the two LC-MS systems for this assay was comparable. In addition the method was successfully applied to evaluate the pharmacokinetic quantification of Vemurafenib in cancer patients treated with Vemurafenib [2]

III.Aim, Objectives and Plan of Work

Aim: Review of literature for Vemurafenib gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs. Literature survey reveals that certain chromatographic methods were reported for estimation of Vemurafenib and single method is available for such estimation by RP-HPLC.Validation is a necessary and important step in both framing and documenting the capabilities of the developed method. Forced Degradation Studies should be carried out to provide evidence on how the quality of pharmaceutical products varies with the time under the influence of environmental factors.

Objectives: To develop new simple, sensitive, accurate and economical analytical stability indicating method for the simultaneous estimation vemurafenib bulk and pharmaceutical dosage form. To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Vemurafenib in bulk and pharmaceutical dosage form.

The plan of the proposed work: -

1. Collection of literature for the selected drug.
2. Extensive literature survey for selection of appropriate solvents to dissolve respective selected drug.
3. Study of drug profile
4. Procurement of samples, standards and other chemicals.
5. Selection of chromatographic conditions
6. Selection of mobile phase
7. Method trials on HPLC by using different solvents and columns.
8. Development of RP-HPLC method which is different from the finished articles.
9. Optimization of the developed method by varying mobile phase conditions, temperature.
10. Validation of the developed method for the following parameters:

11.Forced Degradation Studies

IV.Experimental Work

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.3ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution: Take average weight of the Powder and weight 10 mg equivalent weight of Vemurafenib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Optimization of Mobile Phase: Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Methanol 80:20% v/v) respectively.

Optimization of Column: The method was performed with various C18 columns like Symmetry, Zodiac and Xterra. Symmetry ODS C18 (4.6 x 250mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

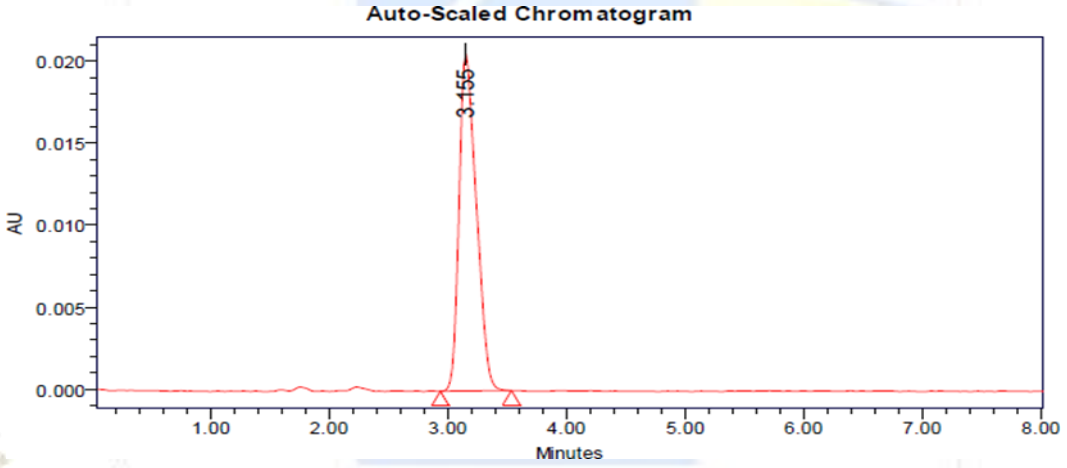


Fig 2: Optimized Chromatogram for Vemurafenib

Table 1: Results of Optimized Chromatogram for Vemurafenib

S.No	Peak name	Rt	Area	Height	USP Tailing	USP plate count
1	Vemurafenib	3.155	225645	20523	1.39	6256

Method Validation:

System suitability

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity study of drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Powder and weight 10 mg equivalent weight of Vemurafenib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:Inject the five replicate injections of standard and inject the three replicate injections sample solutions and calculate the assay.

Linearity

Preparation of drug solutions for linearity:

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Vemurafenib):

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (20ppm of Vemurafenib):

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (30ppm of Vemurafenib):

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (40ppm of Vemurafenib):

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (50ppm of Vemurafenib):

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:Inject each level into the chromatographic system and measure the peak area.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Vemurafenib Product Solution for Precision

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent. For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.45ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Vemurafenib and calculate the individual recovery and mean recovery values.

Limit of detection (LOD) and limit of quantification (LOQ):

Preparation of 0.597µg/ml solution (LOD):

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.Further pipette 0.00597ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of 1.811µg/ml solution (LOQ):

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.Further pipette 0.01811ml of the above Vemurafenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Robustness:

The analysis was performed in different conditions to find the variability of test results.

For preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Vemurafenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. ACN: Methanol was taken in the ratio and 75:25, 85:15 instead of 80:20, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Table-2: Results of system suitability for Vemurafenib

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vemurafenib	3.192	225645	20584	6286	1.38
2	Vemurafenib	3.146	225847	20965	6358	1.39
3	Vemurafenib	3.123	228656	20758	6285	1.41
4	Vemurafenib	3.167	228547	20859	6278	1.40
5	Vemurafenib	3.158	229658	20968	6395	1.42
Mean			227670.6			
Std. Dev.			1810.899			
% RSD			0.795403			

Specificity

Table-3: Results of Assay (Standard) for Vemurafenib

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vemurafenib	3.146	220136	20568	6125	1.36
2	Vemurafenib	3.123	220187	20653	6132	1.38
3	Vemurafenib	3.192	220175	20548	6129	1.34
4	Vemurafenib	3.164	220196	20698	6187	1.35
5	Vemurafenib	3.181	220134	20548	6159	1.35
Std. Dev.			28.91885			
% RSD			0.013135			

Table-4: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Vemurafenib	3.170	224596	20469	1.35	6098	1
2	Vemurafenib	3.174	224658	20489	1.34	6108	2
3	Vemurafenib	3.170	224585	20458	1.35	6107	3

Table-5: Data for Linearity

Concentration (µg/ml)	Average Peak Area
10	78683
20	146545
30	213584
40	279895

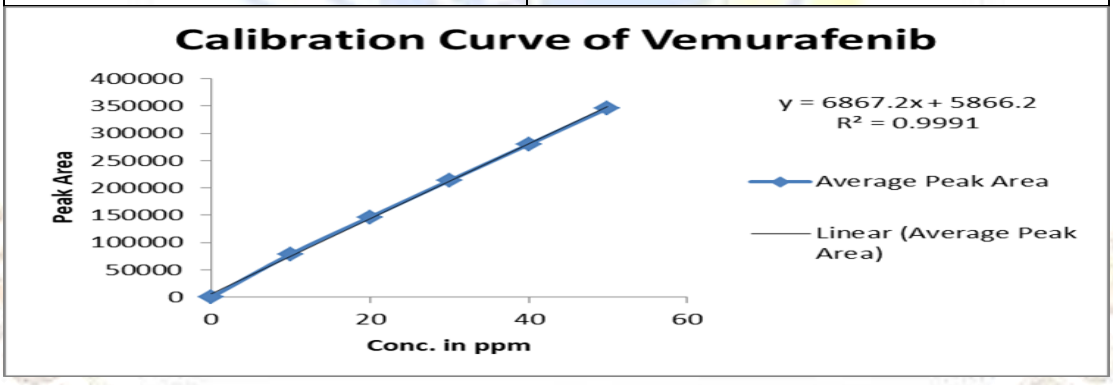


Fig-3: Calibration Curve of Vemurafenib

Precision:

Repeatability

Table-6: Results of method precision for Vemurafenib:

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vemurafenib	3.165	225645	20562	6125	1.36
2	Vemurafenib	3.163	225847	20645	6129	1.36
3	Vemurafenib	3.158	226542	20534	6135	1.35
4	Vemurafenib	3.167	226598	20564	6189	1.36
5	Vemurafenib	3.171	226584	20549	6138	1.35

6	Vemurafenib	3.181	226859	20685	6179	1.37
Mean			226345.8			
Std. Dev			482.1068			
%RSD			0.212996			

Table-7: Results of Ruggedness for Vemurafenib

S.No	**Peak Name**	**RT**	**Area (µV*sec)**	**Height (µV)**	**USP Plate Count**	**USP Tailing**
1	Vemurafenib	3.165	226534	20653	6235	1.35
2	Vemurafenib	3.163	226542	20598	6198	1.36
3	Vemurafenib	30158	225989	20653	6254	1.36
4	Vemurafenib	3.167	226512	20548	6281	1.35
5	Vemurafenib	3.171	226531	20653	6199	1.36
6	Vemurafenib	3.171	225898	20658	6253	1.35
Mean			226334.3			
Std. Dev.			304.2622			
			0.1343			

Table-8: Results of Intermediate Precision Analyst 2 for Vemurafenib

S.No.	**Peak Name**	**RT**	**Area (µV*sec)**	**Height (µV)**	**USP Plate**	**USP Tailing**
1	Vemurafenib	3.173	225487	20542	6253	1.35
2	Vemurafenib	3.134	225484	20532	6098	1.36
3	Vemurafenib	3.161	225364	20541	6254	1.35
4	Vemurafenib	3.174	226513	20534	6235	1.36
5	Vemurafenib	3.199	225487	20549	6199	1.36
6	Vemurafenib	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std. Dev.			553.0524			
% RSD			0.244918			

Table-9: The accuracy results for Vemurafenib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109283.3	15	15.060	100.40%	100.42%
100%	212732	30	30.124	100.413%	
150%	316263.3	45	45.201	100.446%	

Limit of detection for Vemurafenib

LOD= 3.3 × σ / s

Where σ = Standard deviation of the response S = Slope of the calibration curve **Result:**

= 0.597µg/ml

Limit of quantitation for Vemurafenib

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined**LOQ = 10×σ/S**

Whereσ = Standard deviation of the response S = Slope of the calibration curve **Result:**

= 1.811µg/ml

Robustness

Table-10 Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

Forced degradation studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient.

Acid Degradation Studies: To 1 ml of Vemurafenib stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (30µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

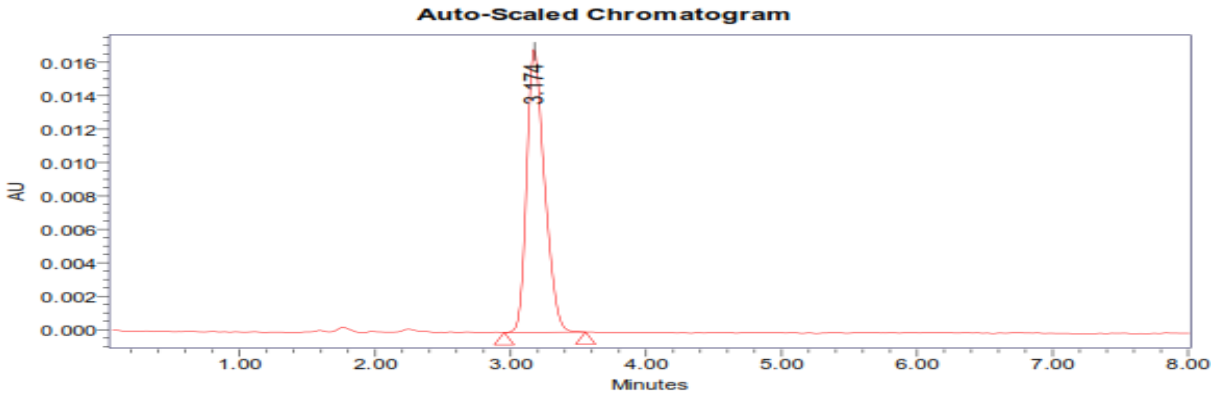


Fig-4: Acid Degradation of VemurafenibChromatogram

Alkali Degradation Studies: To 1 ml of stock solution of Vemurafenib 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCl and makeup to final volume to obtain (30µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. The sample of 20µl was injected into the system, and the chromatograms were recorded to an assessment of sample stability.

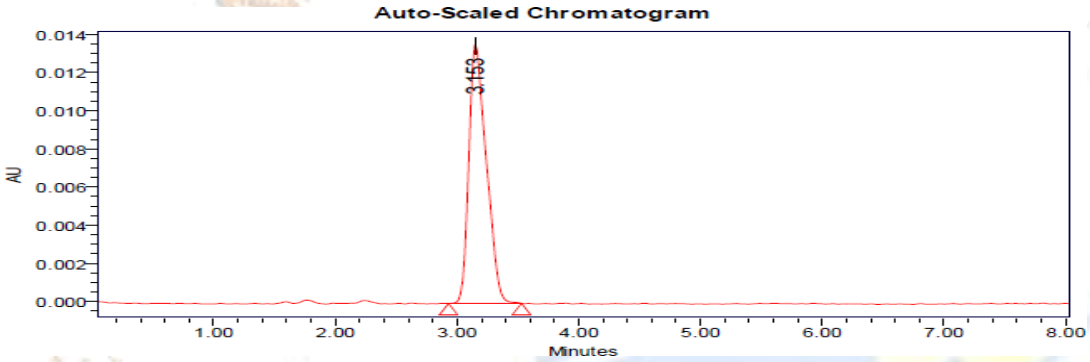


Fig-5: Alkaline Degradation of VemurafenibChromatogram

Oxidation Degradation Studies: To 1 ml of stock solution of Ethinylestradiol and Vemurafenib 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solution was kept for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain (20µg/mL and 30µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

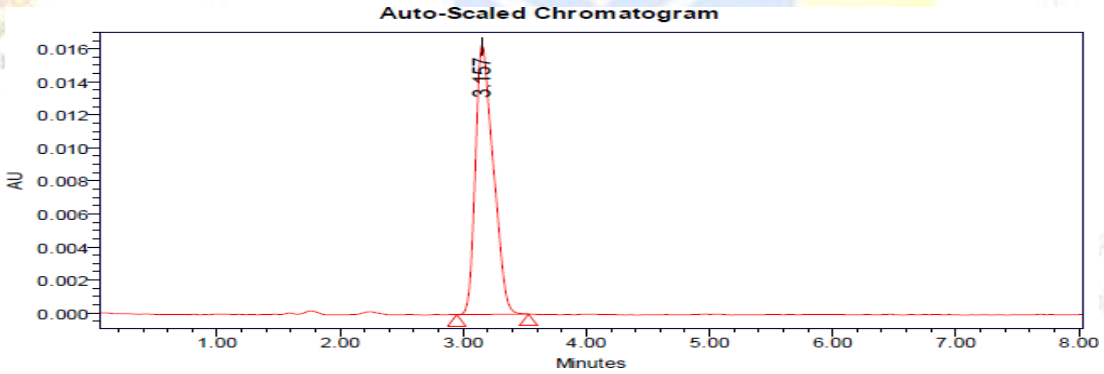


Fig-6: Oxidative Degradation of Vemurafenib Chromatogram

Dry Heat Degradation Studies: The 1 ml of standard drug solution was placed in the oven at 60ºC for 6h to study dry heat degradation. For HPLC study, the resultant solution was makeup to final volume to obtain (30µg/mL) solution. Cool the solution to room temperature and filtered through a 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

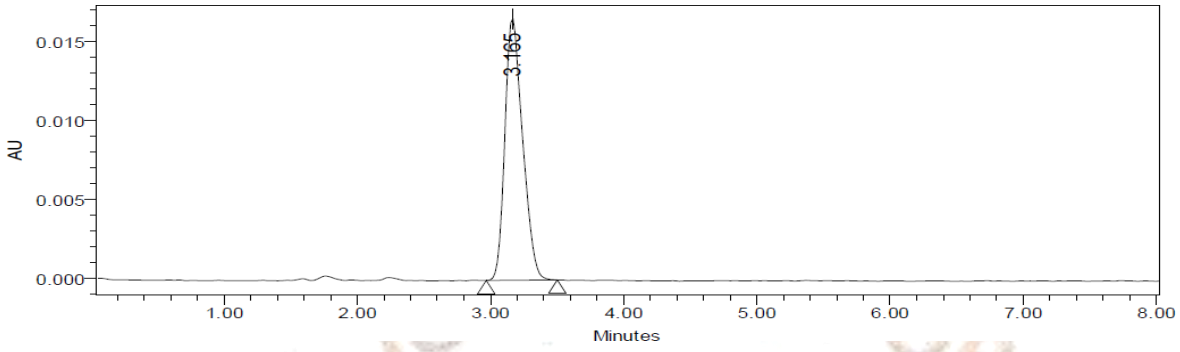


Fig-7: Thermal Degradation of VemurafenibChromatogram

Photo Degradation Studies: The photo stability of the drug was studied by exposing the stock solution to UV light for 1day or 200Watt-hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (30µg/mL) solution and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system.

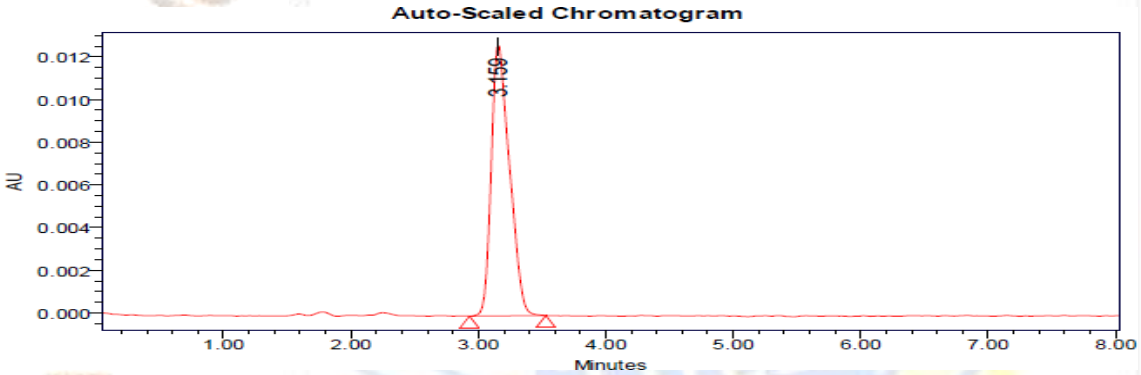


Fig-8: Photolytic Degradation of Vemurafenib Chromatogram

Water Degradation Studies: To 1 ml of stock solution of Vemurafenib, 1 ml of distilled water was added. The solution was kept aside for 30 min at 60 ºC. For HPLC study, the resultant solution was diluted to obtain (30µg/mL) cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded for the assessment of sample stability.

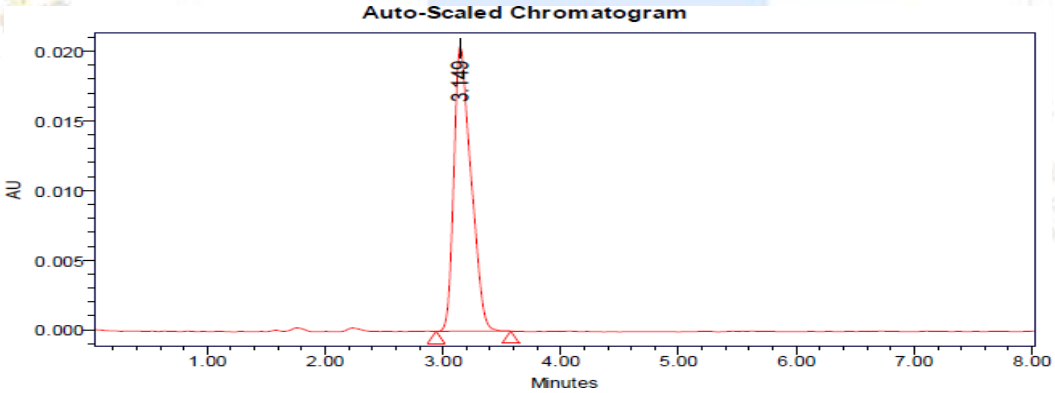


Fig-9: Water Degradation of Vemurafenib Chromatogram

Table-11: Results of Forced Degradation Studies for Vemurafenib

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	225645	0	100%	100%
2	Acidic	190015.65	15.79	84.21	100%
3	Basic	187353.04	16.97	83.03	100%
4	Oxidative	190985.92	15.36	84.64	100%
5	Thermal	183020.65	18.89	81.11	100%
6	Photolytic	181034.98	19.77	80.23	100%

V.Conclusion

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be in UV Spectroscopic Method at 272nm and the peak puritywas excellent. Injection volume was selected to be 20µl which gave a good peak area.The column used for study was Symmetry ODS C18 (4.6 x 250mm, 5µm)because it was giving good peak.Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Acetonitrile: Methanol (80:20% v/v)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.Run time was selected to be 8.0min because analyze gave peak around 3.155 and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range.The analytical method was found linearity over the range of 10-50µg/ml of the Vemurafenib target concentration.The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.The results of the degradation studies indicated the Specificity of the method that has been developed. Vemurafenib was stable in water stress conditions.

VI.References

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