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Research Article

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NEW RVERSE PHASE- H.P.L.C METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF IN VEMURAAFENIB IN FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Vemurafenib in tablet dosage form. Isocratic elution at a flow rate of 0.8ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of methanol, Acetonitrile, THF 65:20:15 v/v/v . The UV detection wavelength was 271nm and 20µl sample was injected. The retention time for Vemurafenib was 6.0 min. 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 Vemurafenib in tablet dosage form.

Key Words: Vemurafenib RP-HPLC, UV detection,271 nm, recovery, precise.

INTRODUCTION

Vemurafenib only works in melanoma patients whose cancer has a V600E BRAF mutation (that is, at amino acid position number 600 on the B-Raf protein, the normal valine is replaced by glutamic acid). About 60% of melanomas have this mutation. Melanoma cells without this mutation are not inhibited by vemurafenib; the drug paradoxically stimulates normal BRAF and may promote tumor growth in such cases.[1,2] In vitro, a melanoma cell line A375 is inhibited by silencing the BRAF gene by short hairpin RNA.[3]. Two mechanisms of resistance to vemurafenib (covering 40% of cases) have been discovered:The cancer cells begin to overexpress a cell surface protein PDGFRB creating an alternate survival pathway.A second oncogene called NRAS mutates, reactivating the normal BRAF survival pathway.[4] At the maximum tolerated dose (MTD) of 960 mg twice a day 31% of patients get skin lesions that may need surgical removal.[5]

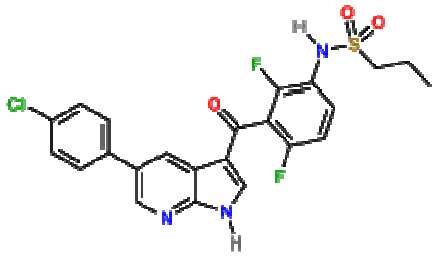


Fig-1 Structure of Vemurafenib

EXPERIMENTAL CONDITIONS

Samples,Standard solutions of Vemurafenib prepared in in 50mL – 100mL brown glass bottles closed with plastic screw closures. The temperature during their storage ranged between 19 and 26°C. Methanol and Acetonitrile(Merck) were of HPLC grade,The HPLC device is PEAK consisted of an LC 7000 pump, a Rheodyne manual sample injector equipped with 20µL loop, a chromatographic column Kromasil® C18, 250 x 4.6mm, particle size 7.5µm (Prochrome), maintained at an ambient temperature, and anUV detector. A suitable flow rate for the mobile 0.8ml/min. Mobile phase (methanol, Acetonitrile, THF 65:20:15 v/v/v) was 0.8mL/min. A wavelength of 271 nm chosen for spectrophotometric detection conforms to an absorption maximum of Vemurafenib in the mobile phase. The injection volume was 20L. Samples of Vemurafenib were diluted 100times before the analyses in the mobile phase.

The Chromatographic analyses were evaluated using Peak software. The chromatographic system was validated by an analysis of solutions with a known analyte concentration. Both the validation results and the results of the analyses of the Vemurafenib samples were statistically

processed by testing for outliers in range, calculation of arithmetic mean and estimation of relative standard deviation . A digital Helico pH meter with a combined Metrohm glass electrode was used for pH measurement.

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility the drug Vemurafenib preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected.The elution of the Vemurafenib from the column was influenced by polar mobile phase. Mixture of was selected as mobile phase and the effect of composition of mobile phase methanol, Acetonitrile, THF 65:20:15 v/v/v were optimized to give symmetric peak with short run time Fig.2. Formulation cheromatogram was showed in Fig.3

Validation of method

Linearity: Five points graphs was constructed covering a concentration range 5-80 ppm (Three independent determinations were performed at each concentration). Linear relationships between the peak area signal of Vemurafenib the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Table.1 Linearity Results

S.NO	Concentration in ppm	Peak Area
1	5	4021
2	10	7589
3	20	17248
4	40	33275
5	80	63258

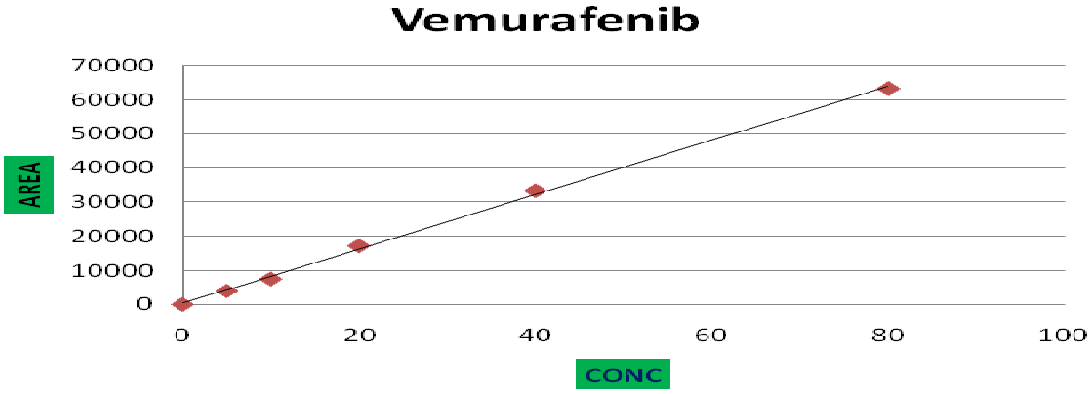


Fig.2 Linearity Curve

Precision

The validated method was applied for the assay of commercial tablets containing Vemurafenib. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on two consecutive days indicated a RSD of 0.875 This indicates good method precision.

Table.2 Precision Results

Injection NO	Concentration of Sample in ppm	Peak Area
1	20	17248
2	20	17301
3	20	17260
4	20	17632
5	20	17371
6	20	17406

Table.3 Formulation Assay

S.NO	Formulation	Dose	Drug Estimated	% of Drug Estimated
1	ZELBORAF	240 mg	238.56	99.41

Stability

The stability of Vemurafenib in standard and sample solutions containing determined by storing the solutions at ambient temperature (20±10^{0}C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that Vemurafenib is stable and standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be

used for the rapid and reliable determination of Vemurafenib in tablet formulation. The results are furnished in Table.4

Fig.3 Chromatogram of Vemurafenib

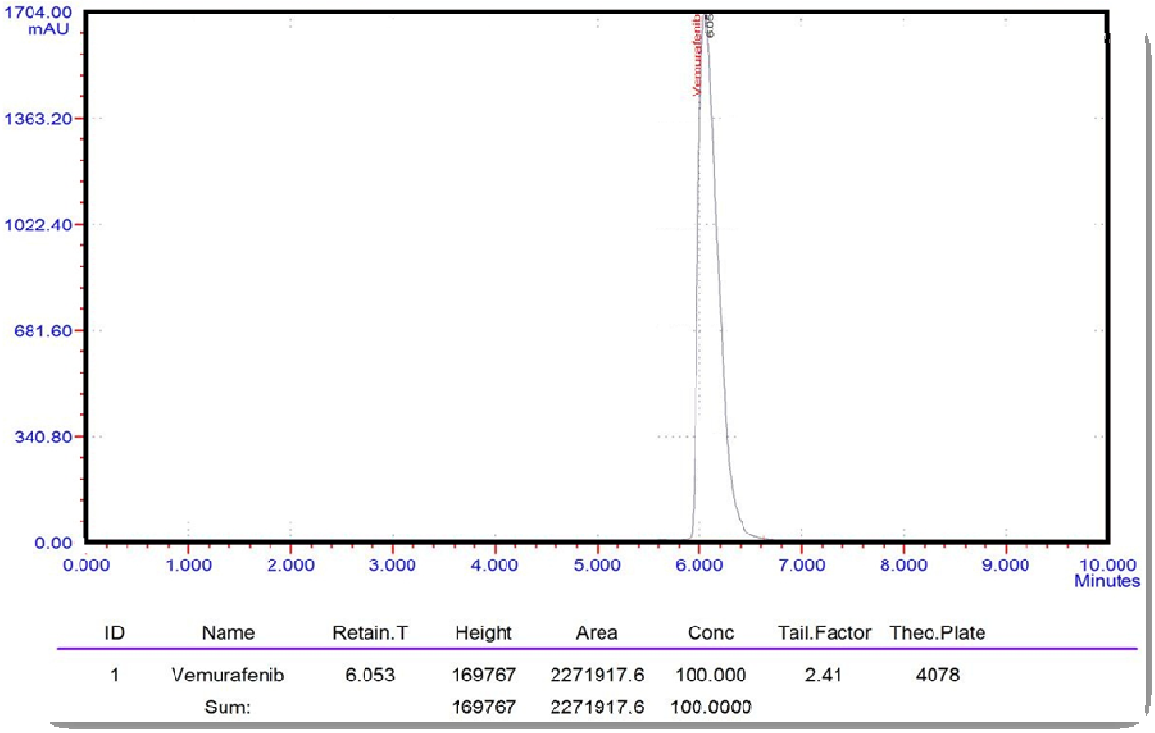
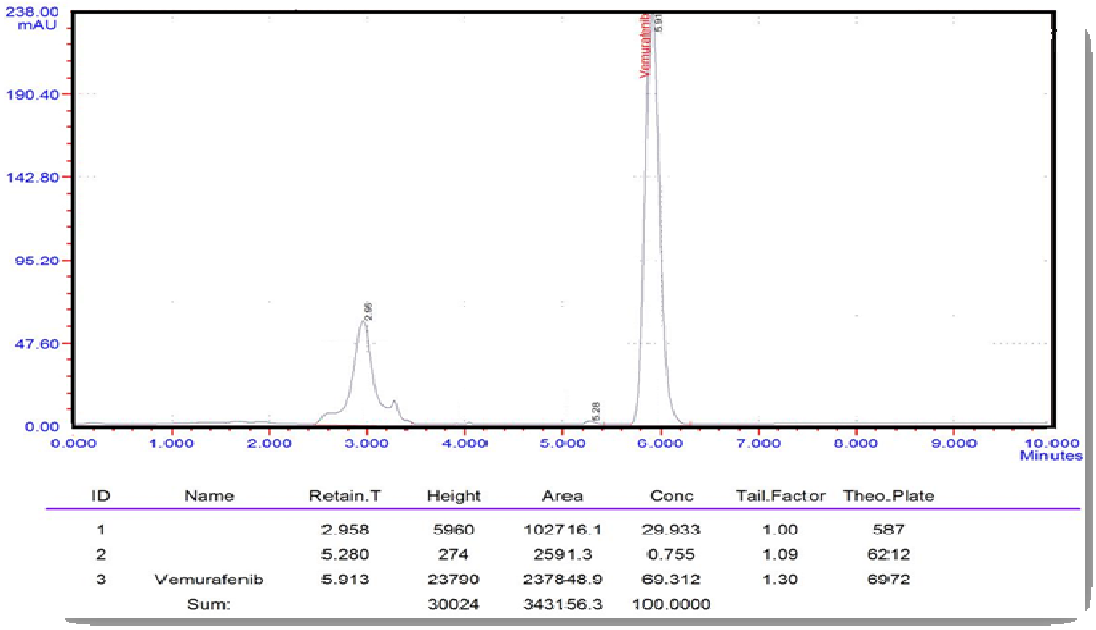


Table-4 System suitability conditions for Vemurafenib

1	Mobile phase	Methanol, Acetonitrile, THF 65:20:15 v/v/v
2	Column	Chromosil C18,250×4.6 mm
3	Mobile phase p H	4.2
4	Flow Rate	0.8ml/min
5	Runtime	10 min
6	Retention Time	6.0 min
7	Linearity range	5-80ppm
8	Slope	791.794
9	Intercept	532.58
10	Correlation coefficient	0.9993
11	% of R.S.D	0.875

Fig.4 Chromatogram of Vemurafenib Formulation



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		2.958	5960	102716.1	29.933	1.00	587
2		5.280	274	2591.3	0.755	1.09	6212
3	Vemurafenib	5.913	23790	237848.9	69.312	1.30	6972
	Sum:		30024	343156.3	100.0000		

Vemurafenib Esitmation in Formulation

Weigh 20 Vemurafenib tablets (Zelboraf-240mg) and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Vemurafenib in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Vemurafenib was measured for the determination. The results are furnished in Table 4.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Vemurafenib in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Vemurafenib in pharmaceutical dosage form.

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