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Development and validation of a new simple and stability indicating RP-HPLC method for the determination of vemurafenib in presence of degradant products

[1]Pawanjeet. J. Chhabda, [1]M. Balaji, [2]Srinivasarao V. and [2]K. M. Ch. Appa Rao

[1]Department of Biochemistry, Ahmednagar College, Ahmednagar, India
[2]Department of Chemistry, Gitam Institute of Science, GITAM University, Visakhapatnam, India

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ABSTRACT

A novel, simple, precise and stability indicating reverse phase high performance liquid chromatography method was developed and validated for the quantitative analysis of Vemurafenib in bulk drug and dosage form using C8 column(150x 4.6, 3.5 µm) with mobile phase consisting of buffer-acetonitrile (50:50 v/v) with a flow rate of 1.0ml/min (UV at 254nm). Linearity was observed over the concentration range of 20-200 µg/ml with r^2= 0.9999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. Vemurafenib was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Vemurafenib is more sensitive towards acidic and alkaline degradation. The method was validated as per ICH guidelines.

Keywords: Vemurafenib.validation.HPLC.Stability indicating

INTRODUCTION

Vemurafenib is a B-Raf enzyme inhibitor developed by Plexxikon and Genentech for the treatment of late stage melanoma. The name vemurafenib comes from V600E mutated BRAF inhibition[14]. Vemurafenib is available as tablets at the dose of 240 mg in the market under the brand name of Zelboraf. Vemurafenib is chemically propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4difluoro-phenyl}-amide with empirical formula is $C_{23}H_{18}CLF_2N_3O_3$ Sand molecular weight 489.9[15].

Various methods in the literatures involve determination of Vemurafenib in human plasma by HPLC [1-2], LCMS/MS [3], pharmacokinetics, pharmacodynamics [4-10]. However no method is available for stability indicating HPLC method of Vemurafenib in bulk drug and pharmaceutical dosage form. In the present work we have developed a new, simple precise and stability indicating method for determination of Vemurafenib in bulk drug and pharmaceutical dosage form.

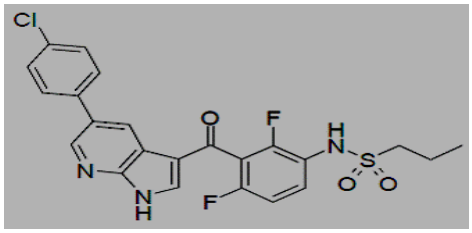


Figure 1: Structure of Vemurafenib

MATERIALS AND METHODS

Chemicals & Reagents

Vemurafenib is available as tablets with brand name ZELBORAF was purchased from local market, containing vemurafenib 240mg. HPLC grade acetonitrile, AR grade ortho phosphoric acid were purchased from Merck, Mumbai. High pure water was prepared by using Millipore Milli-Q plus purification system.

Chromatographic Conditions

A Alliance e2695 separation module (Waters corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. Buffer consisted of 0.1% orthophosporic acid in water (1ml of phosphoric acid in 1000 ml of water). A Zorbax eclipsed XDB C8 (4.6x150) mm,3.5 µm column and isocratic mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The isocratic program was fixed as A: B (50:50v/v). Water and acetonitrile (20:80 v/v) used as diluent. The column oven maintained at 30˚c with 1.0ml flow rate. An injection volume 10µl was used. The elution compounds were monitored at 254 nm.

Preparation of Stock and standard solutions

Accurately 50mg of Vemurafenib standard dissolved in 50ml diluent to get a concentration of 1000µg/ml. Further 10ml of stock solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml.

Preparation of sample (Tablets)

20 tablets of Vemurafenib were powdered and an amount of powder equivalent to 50mg of drug was weighed and transferred to the 50ml flask added 10ml diluent and placed in an ultrasonicator for 10minites made up to the volume with diluent, and filtered through a 0.45µm nylon syringe filter. 10ml of this solution was taken into 100 ml flask and diluted volume with diluent to get concentration 100µg/ml.

Forced Degradation studies

The study was intended to ensure the effective separation of vemurafenib and its degradation peaks of bulk drug and formulation dosage form at the retention time of vemurafenib. Forced degradation studies were performed to evaluate the stability indicating properties of the method [11]

Acid Degradation studies

Acid decomposition was carried out in 0.1N HCL at concentration of 1000µg/ml Vemurafenib and after refluxation for 24hrs at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3(g). The results are tabulated in table 5.

Alkali Degradation studies

Base decomposition was carried out in 0.1N NaOH at concentration of 1000µg/ml Vemurafenib and after refluxation for 24hrs at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3(i). The results are tabulated in table 5.

Oxidation

Oxidation was conducted by using 5%H2O2 solution at room temperature. After 24hrs, 10ml of solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml filtered and injected. The resulting chromatogram is shown in fig.3(k). The results are tabulated in table 5.

Temperature Stress studies

1g of Vemurafenib sample was taken into a petridish and kept in oven at 80˚c for 7 days. 50mg of sample was taken into 50 ml flask diluted volume with diluent, further 10ml to 100ml made up with diluent. The results are tabulated in table 5.

Photo stability

1g of Vemurafenib was taken in to a petridish and kept in photo stability chamber 200 W.hr/m^2 in UV Fluorescent light and 1.2M LUX Fluorescent light. 50mg of sample was taken in 50ml flask, dissolved in diluent, further 10ml in 100ml flask diluted volume with diluent. The results are tabulated in table 5

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

The analytical method conditions were selected after testing the different parameters such as column, wavelength, aqueous and organic phase, buffer concentration, mobile phase ratio, diluent, concentration of analyte, flow and other parameters. Zorbax eclipsed XDB C8 (4.6x150) mm, 3.5 µm column was used because of its high resolution capacity and low degree of tailing. For mobile phase selection, the preliminary trials using different compositions of mobile phases containing water and acetonitrile gave poor peak shape. For improving peak shape instead of water ortho phosphoric acid and acetonitrile (50:50) and thus, better peak shape was obtained. Water and acetonitrile(20:80 v/v) used as diluents because Vemurafenib freely soluble. The detection wavelength was chosen as 254nm for Vemu rafenib because they have better absorption and sensitivity at this wavelength (fig-2). Hence selected method was best among the all trails by many aspects[13].

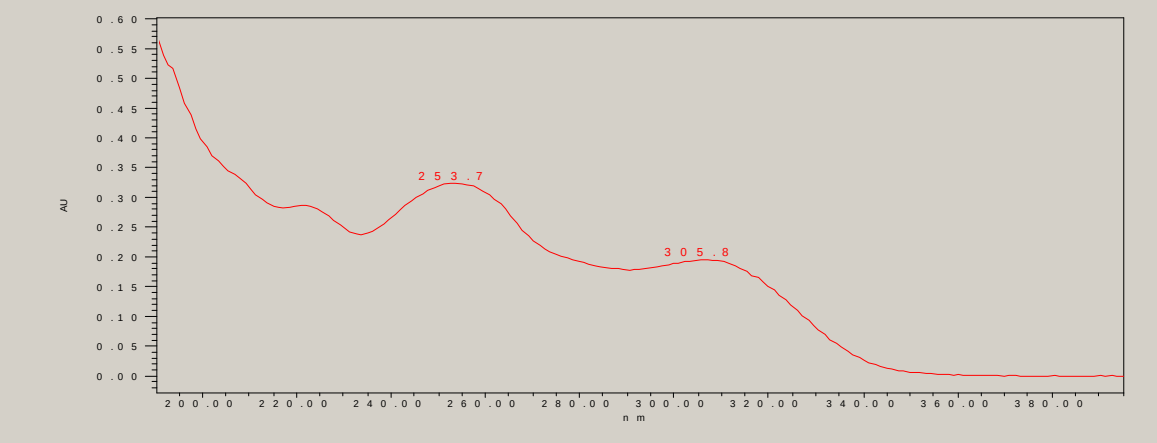


Fig-2 wavelength spectrum of Vemurafenib

Method Validation

The method was validated for the following parameters specificity, linearity, accuracy, limit of detection (LOD), limit of quantitation (LOQ), precision and robustness [12].

Specificity

A study to establish the interference, blank detection was conducted. Diluent was injected as per the test method. Solution of standard and sample were prepared as per test method and injected into the chromatographic system. The chromatograms of blank, standard and sample were shown in the fig a, b, c.

Precision

Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing six sets of sample preparation. Intermediate precision of the analytical method was determined by performing method precision on another day and another analyst under same experiment condition. The % RSD was calculated. The %RSD range was obtained as 0.20 and 0.32 for method

precision and intermediate precision respectively (Table 4) which is less than 2% indicating that the method is more precise.

Accuracy

The accuracy of the method was assessed by determination of recovery for three concentrations (corresponding to 50,100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. The drug concentrations of Vemurafenib were calculated, the percentage recovery was found to be 99.35-99.92% with %RSD 0.03 - 0.16(<2.0%) indicating that the method is more accurate (table 2)

Linearity

The linearity plot was prepared with six concentration levels (20, 40, 80,100,120 and 150 µg/ml of Vemurafenib). These concentration levels were respectively corresponding to 20, 40, 80,100,120 and 200 % of test solution concentration. The results obtained are shown in table 1. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure 4).

Robustness

Robustness of method was checked by making slight deliberate changes in chromatographic conditions like flow rate (±0.1 ml/min) mobile phase composition and column temperature (±5˚c). The results are tabulated in table 3.Under all the deliberately varied chromatographic conditions, the reproducibility of results was observed to be reasonably good. Hence the proposed method has good robustness for the assay of vemurafenib in bulk and dosage forms.

LOD and LOQ

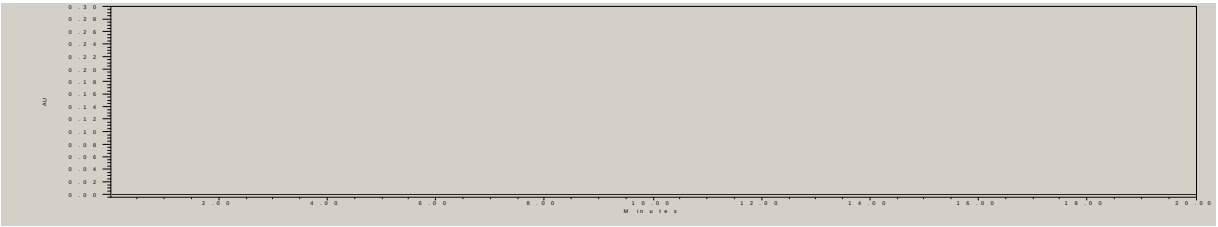
The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of test solutions of known concentrations within the linearity range. Precision study was also carried out at the LOQ level by injecting six pharmaceutical preparations. The LOD and LOQ were to be 0.16µg/ml and 0.55µg/ml respectively. The %RSD value was noticed to be less than 1.0% at LOQ concentration level.

Solution stability and Mobile phase stability

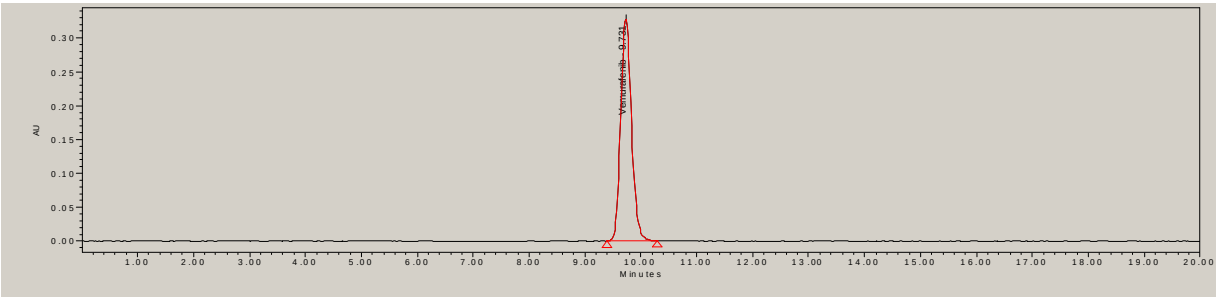
Solution stability checked for stability of standard and sample solutions. Solution stability checked at each interval initial 2,4,6,8,12,16,20 and 24 hours. For standard solution stability and sample solution stability %assay value calculated at each interval. %RSD (NMT 2.0%) between initial assay value and assay value obtained at predetermined time interval calculated.

Forced Degradation Studies

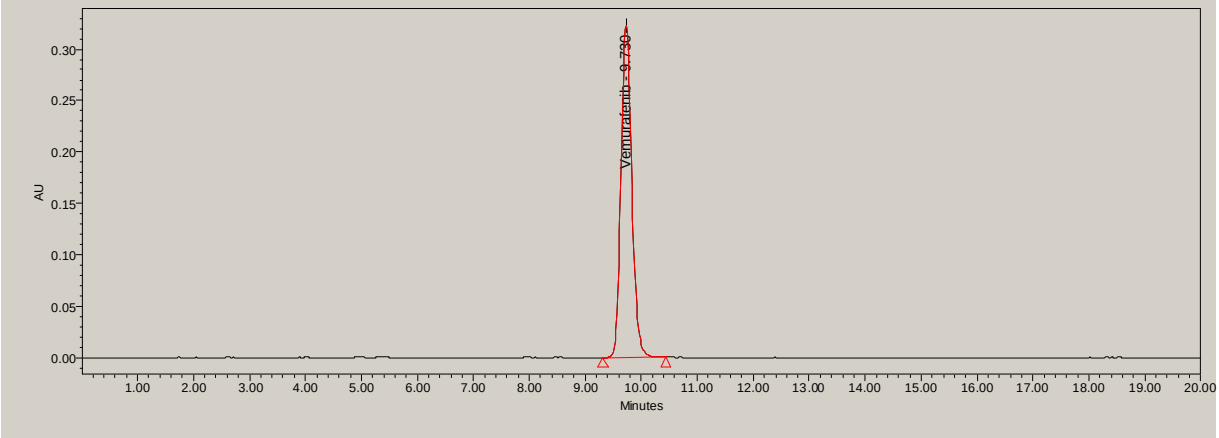
Stress studies on vemurafenib were carried out under oxidation, thermal stress, photolysis, acid and alkali hydrolysis conditions. Significant degradation was observed in acid (fig 3g) and base (fig 3i) of vemurafenib . There was no significant degradation of vemurafenib upon exposure to dry heat at 80˚c for 7days and photolysis total impurity increased to 0.15% and 1.22%. In peroxide oxidation (fig 3k) no significant change was observed, which indicated that the drug was stable against these stress conditions. The developed method revealed that there was no interference from the impurities, degradation products and excipients to determine the assay of drug substance in pure and pharmaceutical formulation.



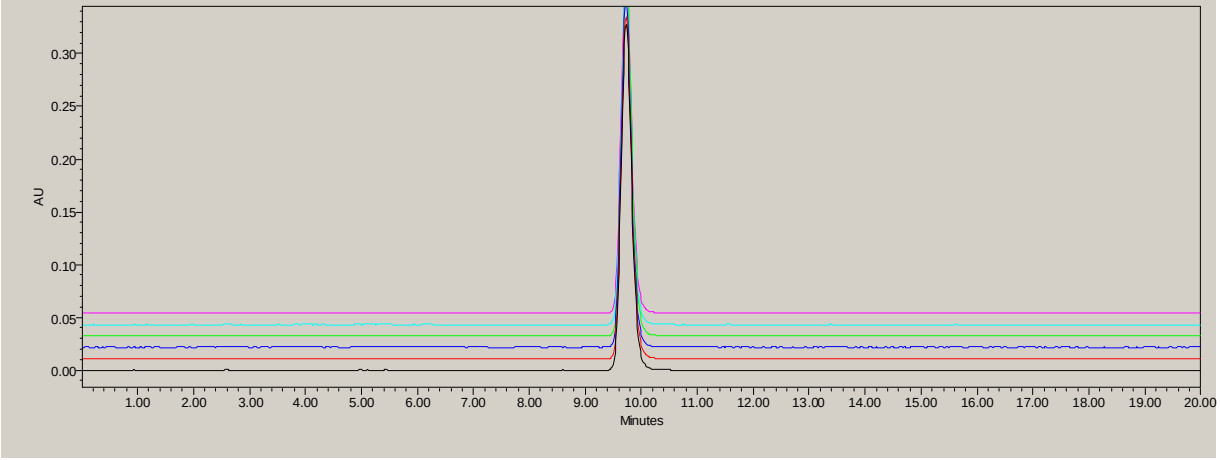
(a)


Vemurafenib - 9.731
AU
Minutes

(b)


Vemurafenib - 9.730
AU
Minutes

(c)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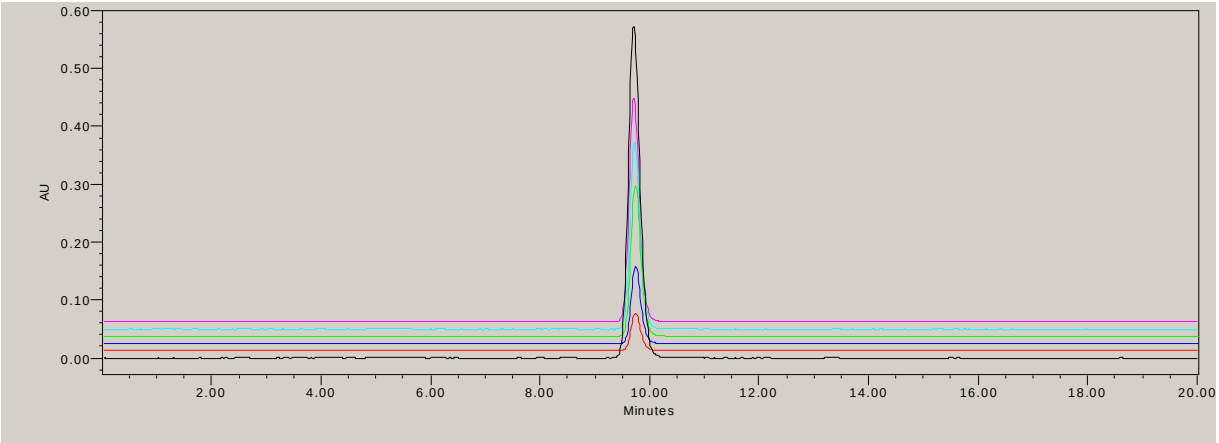
(d)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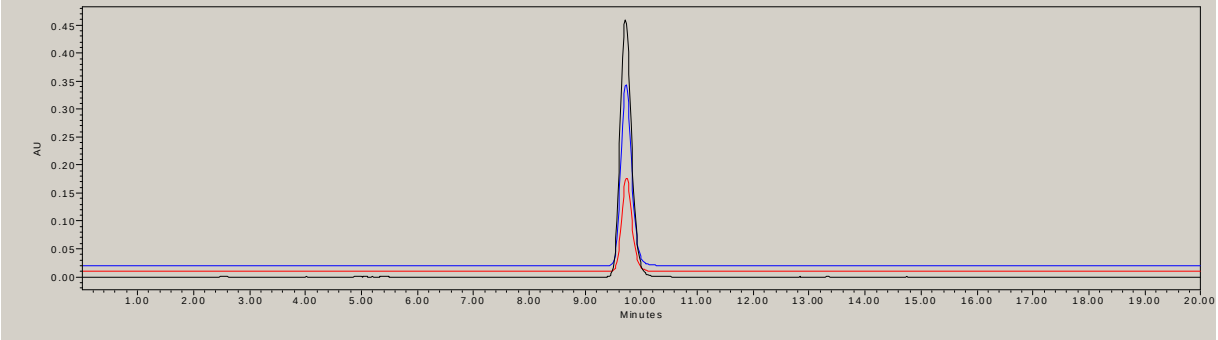
(e)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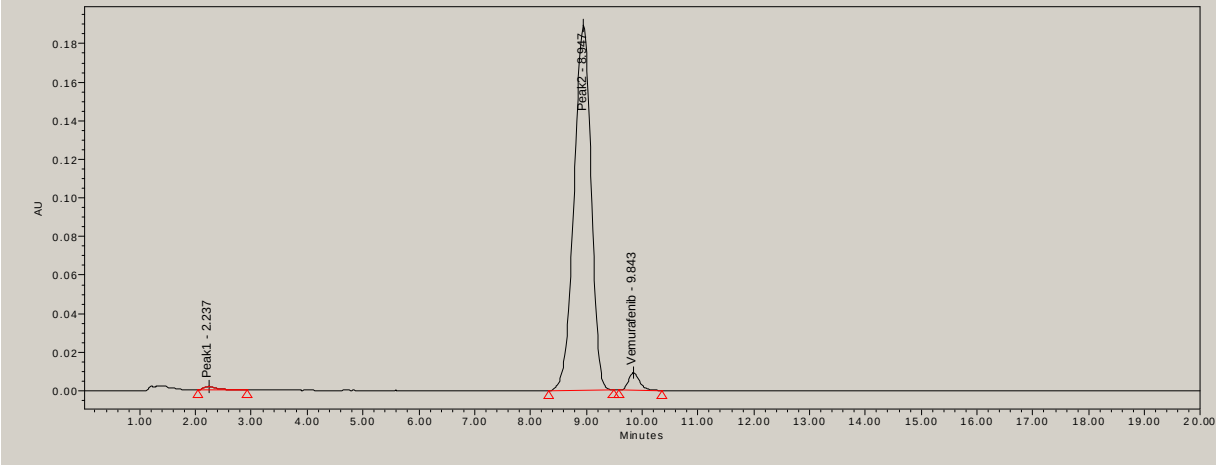
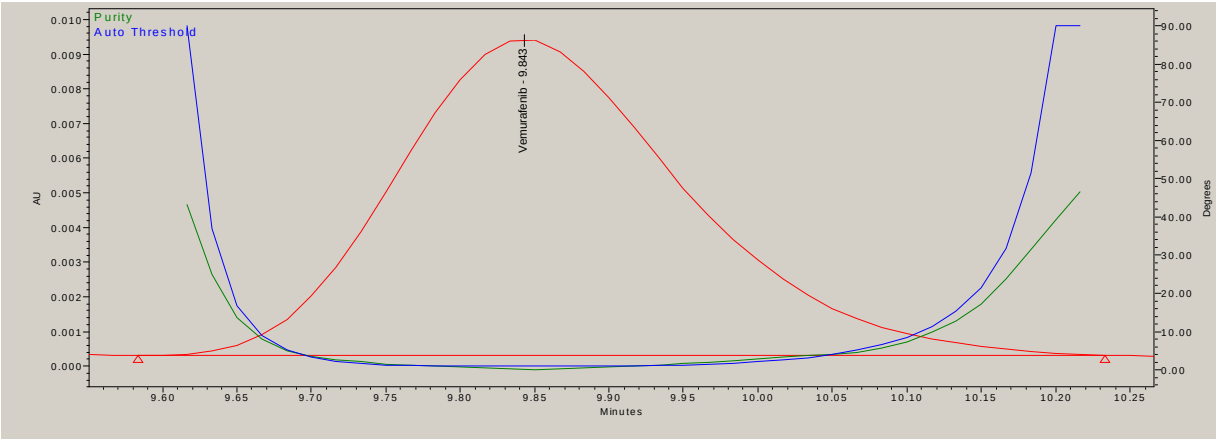
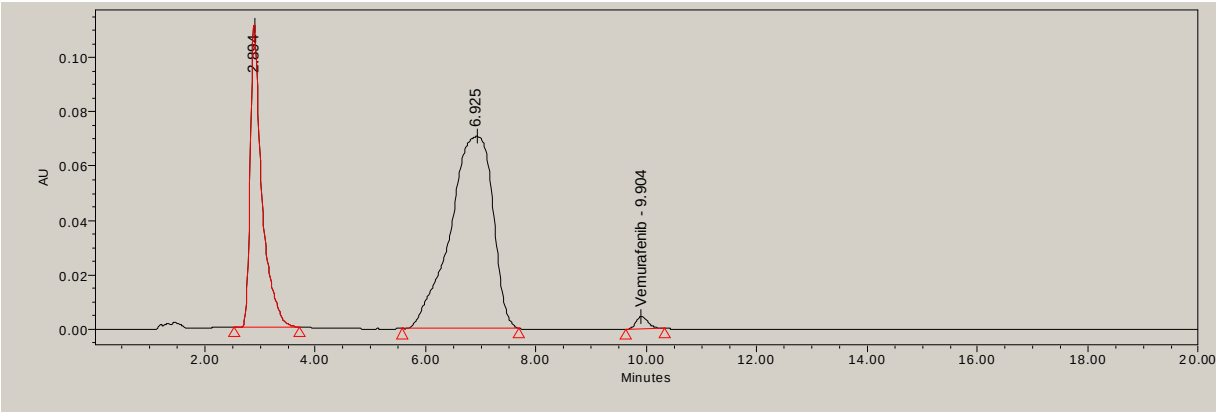
(f)


Peak1 - 2.237
Peak2 - 8.947
Vemurafenib - 9.843
AU
Minutes

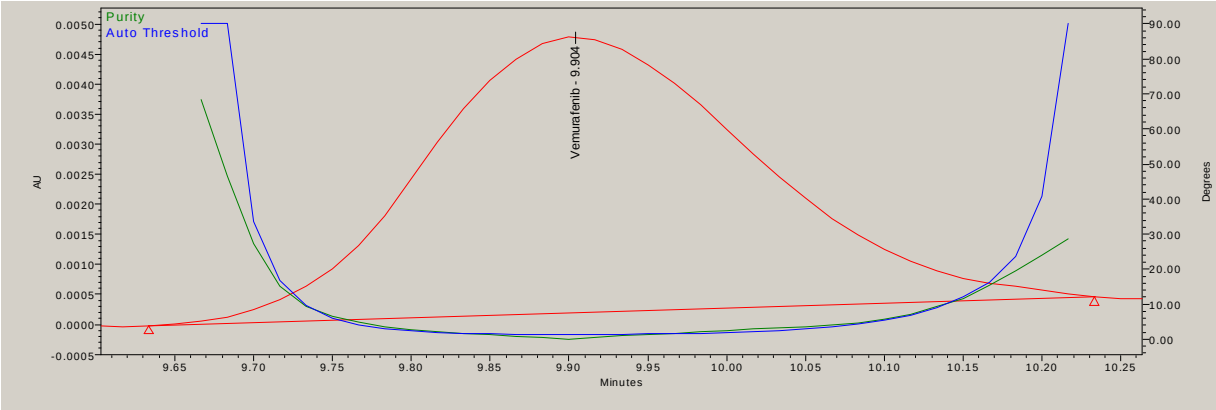
(g)


Purity
Auto Threshold
Vemurafenib - 9.843
AU
Degrees
Minutes

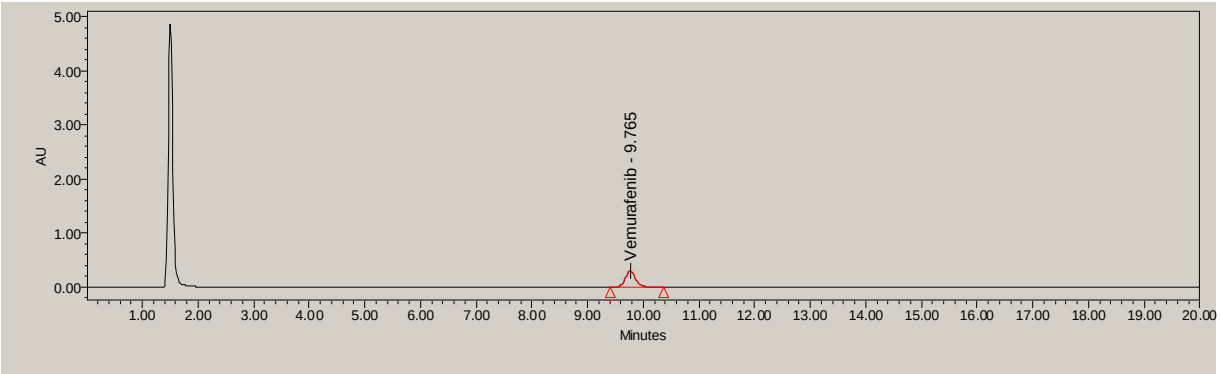
(h)


2.884
6.925
Vemurafenib - 9.904
AU
Minutes

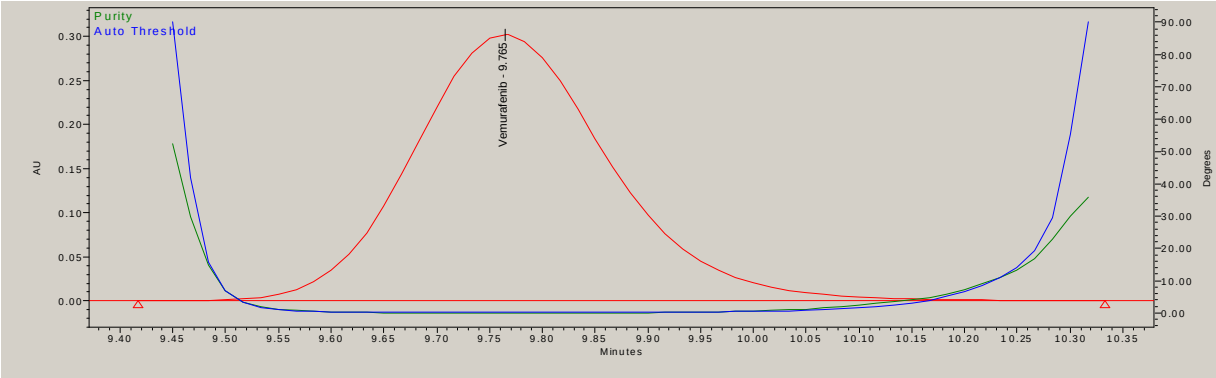
(i)


Purity
Auto Threshold
Vemurafenib - 9.904
AU
Degrees
Minutes

(j)



(k)



(l)

Fig-3 Typical chromatograms of (a) Blank (b) Standard (c) Sample (d) precision injections (e) Linearity injections (f) Accuracy injections (g) Acid sample (h) Purity plot of Acid (i) Base sample (j) Purity plot of Base(k) Peroxide sample(l) Purity plot of Peroxide

Fig-4 Linearity of Vemurafenib

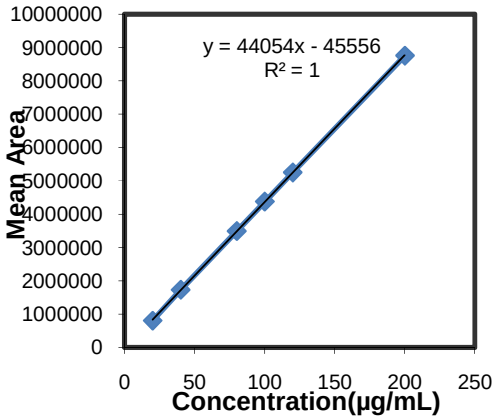


Table-1 Results for linearity of Vemurafenib

Linearity level	%Level	Area
1	20	804710
2	40	1729981
3	80	3489743
4	100	4374679
5	120	5248614
6	200	8749321
Correlation co-efficient		0.999978
	intercept	-45556.5
	slope	44054.26

Table-2 Recoveries study for Vemurafenib

Accuracy (Recovery) study							
Accuracy Level	**Set No**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
50%	1	50.24	49.89	99.3	99.35	0.16	0.16
	2	50.14	49.9	99.52			
	3	50.26	49.87	99.22			
100%	1	100.35	100.29	99.94	99.88	0.16	0.16
	2	100.1	100.09	99.99			
	3	100.18	99.88	99.7			
150%	1	150.18	150.04	99.9	99.92	0.03	0.03
	2	150.05	149.99	99.96			
	3	150.24	150.23	99.99			

Table -3 Robustness results for Vemurafenib

Robust conditions	variation	Retention time(min)	USP Tailing	USP Plate count
Flow	0.9ml	10.68	1.13	12154
	1.0ml	9.74	1.11	12453
	1.1ml	8.8	1.06	12675
Temperature	25˚c	10.25	1.12	12235
	30˚c	9.74	1.11	12453
	35˚c	9.02	1.08	12865
%Acetonitrile	55	8.25	1.06	12875
	50	9.74	1.11	12453
	45	10.9	1.13	12087

Table-4 Precision results for Vemurafenib

Study	Set no	Assay (%)	Mean assay(%)	Stdev	RSD%
Method precision	1	99.9	99.93	0.21	0.2
	2	99.75			
	3	100.2			
	4	100.14			
	5	99.69			
	6	99.89			
Intermediate precision	1	99.58	99.76	0.32	0.32
	2	100.2			
	3	99.7			
	4	99.36			
	5	99.65			
	6	100.08			

Table-5 forced degradation results for Vemurafenib

Stress condition	Drug recovered (%)	Drug decomposed (%)
Standard drug	100	
Acid degradation	10.24	89.76
Alkali degradation	8.90	91.10
Oxidation degradation	100	0.00
Thermal degradation	99.85	0.15
Photolytic degradation	98.78	1.22

CONCLUSION

A new reverse phase HPLC method for the quantitative analysis of Vemurafenib in bulk and pharmaceutical dosage forms is established. This method is a new, simple, precise, linear, accurate and specific. This method is free from interference of the other active ingredients and additives used in the formulation. Degradation impurities did not interfere with the retention time of Vemurafenib, and method is thus stability indicating.

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