RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYISIS OF VENDETANIB IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of VENDETANIB in tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Acetonitrile:ethylalchol:$NH_4H_2PO_4$ 60:25:15(v/v/v). The UV detection wavelength was at 219nm.Linearity was observed in concentration range of 10-60ppm. The retention time for VENDETANIB was 2.1 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of VENDETANIB in pharmaceutical dosage forms.

KEY WORDS: Vendetanib, HPLC, Development, 219nm.

INTRODUCTION:

Vandetanib is an antagonist of the vascular endothelial growth factor receptor (VEGFR) and the epidermal growth factor receptor (EGFR).[1] It is a tyrosine kinase inhibitor, being developed by AstraZeneca.It has a third target: inhibiting RET-tyrosine kinase activity, an important growth driver in certain types of thyroid cancer.

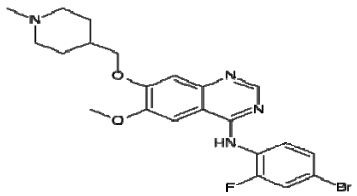

Figure 1: Stricture of Vandetanib

It is a medication currently undergoing clinical trials as a potential targeted treatment for non-small-cell lung cancer. There have been some promising results from a phase III trial with docetaxel.[5] There have also been ambivalent results when used with pemetrexed[6] Another trial with docetaxel was recruiting in July 2009.[7] AstraZeneca withdrew EU regulatory submissions for Zactima in October 2009 after trials showed no benefit when the drug was administered alongside chemotherapy.[8]

EXPERIMENTAL

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile, ammonium acetate which are of HPLC grade were purchased from E.Merck,

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Gemini C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with , Acetonitrile:ethylalchol:$NH_4H_2PO_4$ 60:25:15(v/v/v). was selected with a flow rate of 2.1ml min^{-} The detection wavelength was set at 219nm with a runtime of 5 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100mg of VENDETANIB was weighed and transferred (working standard) into a 100ml volumetric flask. The diluent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 10 to 60ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of VENDETANIB were crushed to give finely powdered material. Powder equivalent to10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 30 ppm

S.NO	TEST	RESULT
	H.P.L.C CONDITIONS	
1	ELUTION	ISOCRATIC
2	A.P.I CONC	30ppm
3	MOBILE PHASE	Acetonitrile:ethylalchol:$NH_4H_2PO_4$ 60:25:15(v/v/v)
4	PH	5.8
5	COLUMN	C18
6	WAVE LENGTH	219
7	FLOW	1ml\min
8	RUNTIME	5 mins
9	RETENSION TIME	2.1
10	AREA	15429

Table-1 chromatographic conditions for Vendetanib

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability

Lineariy

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of VENDETANIB in the mass concentration range of 10ppm to 60ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of VENDETANIB was obtained by plotting the peak area ratio versus the applied concentrations of VENDETANIB. The linear correlation coefficient was found to be 0.999

S.NO	CONC	AREA
1	10ppm	5073
2	20ppm	10216
3	30ppm	15429
4	40ppm	20374
5	50ppm	25618
6	60ppm	30475

.Table 2: Linearity of VENDETANIB

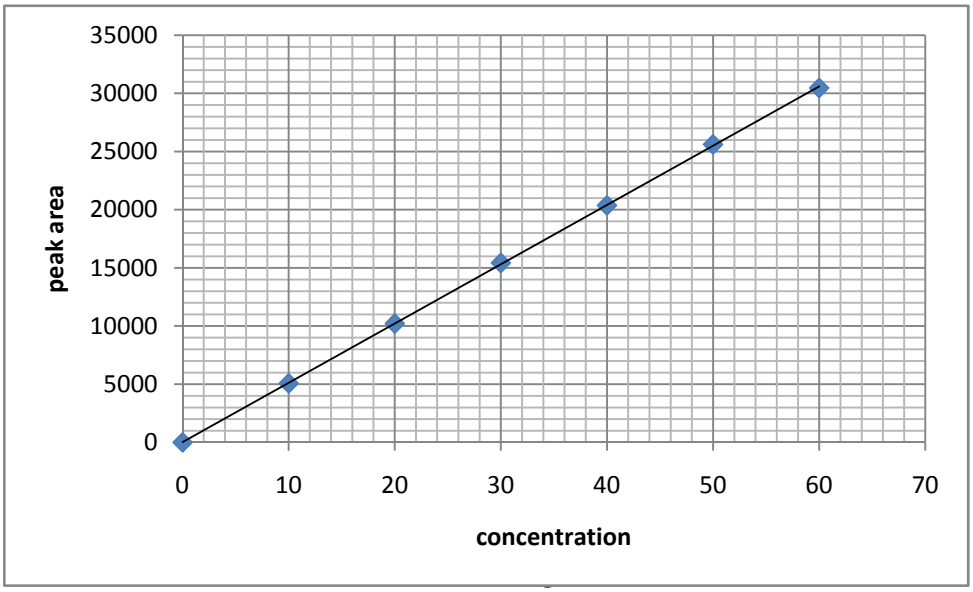

Figure 2: Calibration curve of VENDETANIBS

Drug	VENDETANIB
Concentration range	10-60ppm
Slope (m)	509.5464
Intercept (b)	25.72
Correlation coefficient	0.999
% RSD	Intra day-0.75 Interday-0.92

Table.3 Linear Regression Data for Calibration curve

Precision

Repeatability of the method was checked by injecting replicate injections of 45 ppm of the solution for six times on the same day as intraday precision study of VENDETANIB and the RSD was found to be 0.75for intraday and 0.92for interday

INJECTION	CONCENTRATION	INTRA DAY	INTER DAY
1	30ppm	15384	15419
2	30ppm	15211	15564
3	30ppm	15492	15492
4	30ppm	15505	15835
5	30ppm	15484	15521
6	30ppm	15327	15520
	RSD	0.75	0.92

Table 4: Precision parameters of VENDETANIB

Accuracy

The accuracy of the method was determined by calculating recovery of VENDETANIB by the method of standard addition. Known amount of VENDETANIB was added to a pre quantified sample solution and the amount of VENDETANIB was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of VENDETANIB was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery was calculated and the average recovery was found to be 99.52%

SPECIFICITY

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.05ppm and 0.02ppm respectively as per ICH guide-lines.Results are shown in table 5.

Parameter	Measured
LOD	0.02ppm
LOQ	0.05ppm

Table 5: Results of LOD and LOQ.

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. Results of Robustness are shown in table 6.

Parameter	Modification	Peak Area	% of change
Standard	No change	15429	…….
M.PHASE	Acetonitrile:ethylalchol:$NH_4H_2PO_4$ 55:35:10(v/v/v)	15345	0.994
PH	5.4	15532	1.00
WAVELENGTH	219nm	15299	0.991

Table 6: Robustness results

System Suitability Parameter:

System suitability tests were carried out on freshly prepared standard stock solutions of VENDETANIB and it was calculated by determining the standard deviation of VENDETANIB standards by injecting standards in six replicates at 6 minutes interval and the values were recorded inTable7.

Parameters	Values
λ max (nm)	219nm
Beer's law limit (µg/ml)	10-60ppm
Correlation coefficient	0.999
Retention time	2.1min
Limit of detection	0.02ppm
Limit of quantification	0.05ppm

Table7: System suitability parameters of VENDETANIB

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug VENDETANIB being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile:ethylalchol:$NH_4H_2PO_4$ 60:25:15(v/v/v). The retention time of VENDETANIB was found to be 2.1min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 7. The high percentage of recovery of VENDETANIB was found to be 99.52 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of VENDETANIB in tablet formulation. The result for VENDETANIB was comparable with a corresponding labelled amount . The absence of additional peaks indicates no interference of the excipients used in the tablets.

Formulation	Dosage	Sample concentration	Amount estimated	%Estimation
CAPRELSA	300mg	30ppm	29.89	99.633

Table 8: Formulation results of VENDETANIB

CONCLUSION

A validated RP-HPLC method has been developed for the determination of VENDETANIB in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 5 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of VENDETANIB in pharmaceutical dosage form.

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