Original Research Article

A simple, accurate and efficient High performance Liquid chromatographic method for determination of Vandetanib in Bulk and in Pharmaceutical Forms

Abstract

A simple, selective, linear, precise and accurate isocratic RP-HPLC method was developed and validated for rapid assay of Vandetanib, an anticancer drug, in both bulk and tablet dosage form. Elution at a flow rate of 1ml/min was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of acetonitrile, water and orthophosphoric acid in the ratio of 90:08:02 (v/v/v). Linearity was observed in concentration range of 50-200 ppm. The retention time for Vandetanib was 3.326 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Vandetanib in pharmaceutical dosage forms. Moreover the detection alone was also verified through LC-MS of the Vandetanib drug using ESI method which provides future scope for study of this drug using LC-MS method also.

Key words: Vandetanib, Acetonitrile, orthophosphoric acid, RP-HPLC

1. Introduction:

Vandetanib, an anti-cancer drug, inhibits new blood vessels growth and thereby prevents tumors. It is a potent multi-kinase inhibitor[1][2]. As it can cause a change in the electrical activity of the heart, called QT prolongation, leading to irregular heartbeats it is restricted for use in some patients. Moreover having a half-life of 19 days on an average in blood plasma, it has been a matter of concern in analysis. Few analytical or complex methods have been reported for the determination of Vandetanib in samples using chromatographic[3][4][5][6][7][8][9] and other methods[10][11][12][13][14]. But limited or no simple, precise method to determine it in pure drug and pharmaceutical dosage forms is available. Hence the present investigation has been carried out to develop a simple RP-HPLC method for the analysis of Vandetanib in bulk form and apply the same to determine the drug in pharmaceutical forms.

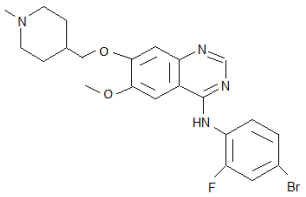


Figure 1: Structure of Vandetanib

2. Materials and Methods:

2.1 Materials

2.1.1 Instrumentation

Liquid chromatography was performed on Gemini C18 5µm, (250×4.6 mm) column, using Agilent 1100 series binary pump with chemstation controller. The mobile phase consists of acetonitrile, water and orthophosphoric acid in the ratio of 90:8:2 (v/v/v) at a flow rate of 1 ml/min.

The liquid chromatographic system was later coupled to Agilent 1100 series LC-MSD equipped with electro spray ionization with the optimized instrumental settings. The other conditions of the mass detector includes fragmentor voltage of 200 volts, capillary voltage of 4000 volts, pressure of 35 psi, with the dry gas flow of 8 L/min and temperature at 300°C. Agilent 1100 chemstation data software was used for data integration.

2.1.2 Chemicals and Solvents

Acetonitrile and orthophosphoric acid of HPLC grade was purchased from E. Merck, Mumbai, India. HPLC grade water was obtained by double distillation and purification through Milli-Q water purification system. The pure form of the drug was procured from V.V Med, Jeedimetla, Hyderabad, India along with the sample under study.

2.1.3 The Mobile phase

The mobile phase consisting of acetonitrile, water and orthophosphoric acid in the ratio of 90:08:02 (v/v/v) was used for the elution.

2.1.4 Standard solution of the drug

Accurately transferred 10 mg of Vandetanib working standard into a 100 ml volumetric flask and dissolved in acetonitrile and made the final volume with water and acetonitrile (1:1) to give 100 ppm solution of Vandetanib. The standard solution was stored properly and later used for analysis.

2.1.5 Sample solution

The tablet forms of Vandetanib were powdered to a fine form and then powder equivalent to 10 mg of the drug was dissolved in 5 ml of the mobile phase taken in 10 ml volumetric flask. After dissolution the solution was filtered through Ultipor Nylon 6, 6 membrane sample filter paper and the filtrate was adjusted to the mark with the same solvent to obtain a concentration of 100 ppm.

2.2 METHOD DEVELOPMENT

A suitable RP HPLC method development involves selection of the appropriate wavelength, solvent, stationary and mobile phases. In order to establish these requirements, a systematic study on the effect of various factors involved was undertaken by varying each of them keeping all other conditions constant.

2.2.1 Detection of wavelength

The wavelength of maximum absorbance was recorded on an UV spectrophotometer using a solution of the drug and found to be 243 nm which was subsequently used for detection of the drug.

2.2.2 Choice of stationary phase

When a number of trials were performed using different octadecyl columns of various types and configurations from different manufacturers an Inertsil ODS C-18 5µm column having 250 x 4.6mm internal diameter gave the expected separation with good peak shapes. Hence it was chosen for the method development.

2.2.3 Selection of the Mobile phase

A number of experiments were carried out by varying the commonly used solvents and their compositions in order to get sharp peak and base line separation of the component under study. An ideal separation was achieved with mobile phase containing acetonitrile, water and orthophosphoric acid in the ratio of 90:8:2 (v/v/v). The drug molecule was tuned in negative mode of ionization as it gave better sensitivity with more reproducibility as stated in the instrumental section above. This was finally selected as it gave a well-defined chromatographic peak with better resolution, base line separation and low tailing factor.

2.2.4 Flow rate

An effective flow rate is one that is minimum with a short run time which can minimize the usage of solvents. The optimum flow rate of 1.0 ml/min was attained by varying it between 0.5–1.5 ml/min. This was ideal for the successful elution of the analyte.

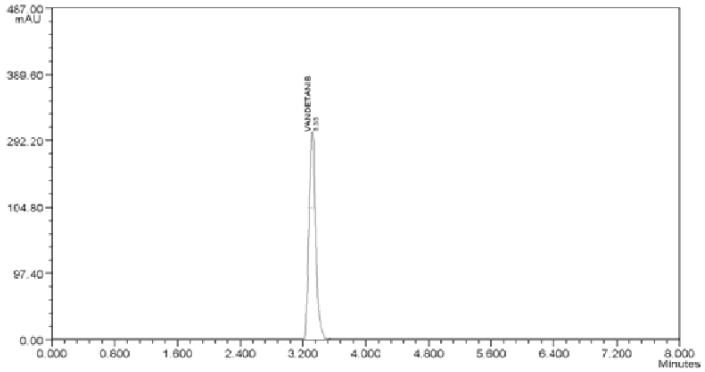
2.2.5 Optimized LC-MS conditions

Optimization of mobile phase was performed based on chromatographic separation, peak shape and peak area obtained. The composition, pH and flow rate of the mobile phase and mass spectrometric conditions were changed to optimize the separation conditions. On the above proceedings, the Chromatographic conditions thus optimized are shown in Table 1.

TABLE 1: Optimized chromatographic conditions

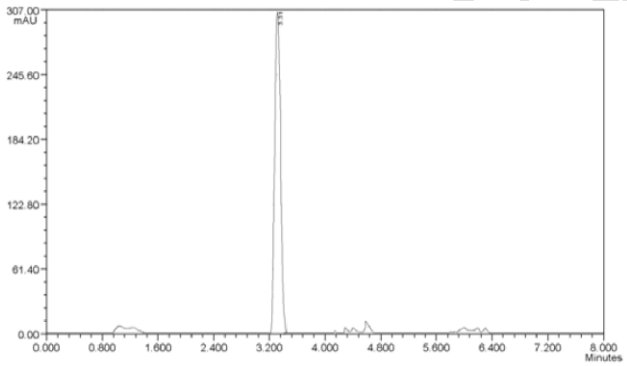
S.No.	Parameter	Condition
1	Mobile phase	Acetonitrile : Water : 0.1% OPA (90:8:2)
2	Pump mode	Isocratic
3	Mobile phase pH	4.1
4	Diluent	Mixture of acetonitrile and water
5	Column	Gemini C18 5µm, (250×4.6 mm)
6	Wavelength	243 nm
7	Injection Volume	20 µl
8	Flow rate	1.0 ml/min.
9	Run time	5 min.
10	Retention Time	3.326 min.
11	Standard concentration	100 ppm
12	Peak area	185676.3
13	Theoretical plates	6018.81
14	Pump pressure	4.9

These conditions were maintained for the determination of Vandetanib in bulk and pharmaceutical forms. When blank solution containing only the mobile phase without the drug was injected, no peak was obtained. The chromatograms of standard and tablet sample are shown in Figure 2 and 3 respectively. The mass spectrum recorded correspondingly is shown in figure 4.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	VANDETANIB	3.326	30580	185676.3	100.000	1.50	6019
	Sum:		30580	185676.3	100.0000		

Figure 2: Chromatogram of standard solution



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		3.315	30739	185134.9	100.000	1.15	6037
	Sum:		30739	185134.9	100.0000		

Figure 3: Chromatogram of formulation

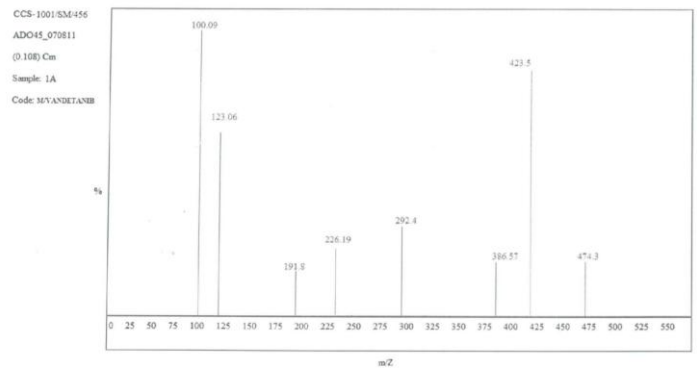


Figure 4: Mass spectra of Vandetanib

3. RESULTS AND DISCUSSION

The experimental method developed above was employed for its subsequent validation and determination of Vandetanib in bulk and pharmaceutical forms. The following results were obtained correspondingly.

Validation of a proposed analytical method to determine the assay should meet the requirements for the intended analytical application as per ICH guidelines. The typical analytical parameters used in validation of the assay include Precision, Accuracy, Linearity, Robustness, Limit of detection, Limit of Quantification, Selectivity or Specificity.

3.1 Linearity

The linearity of calibration curve for Vandetanib was assessed at seven concentration levels in the range of 50-200 ppm in bulk samples. Peak area ratios for each solution against its corresponding concentration were measured and the calibration curve was obtained from the least-squares linear regression presented with their correlation coefficient. The results obtained indicate a linear relationship between peak response and concentration of Vandetanib in the range of 50-200 ppm as shown in figure 5. Linearity results obtained are presented in Table 2.

TABLE 2: Linearity results

Level	Concentration of Vandetanib (in ppm)	Mean peak area
1	50	95444.8
2	75	154956.3
3	100	185676.3
4	125	240639.5
5	150	294114.6
6	175	353703.0
7	200	395479.3
	Slope	2008
	Intercept	-5361
	Correlation coefficient	0.9961

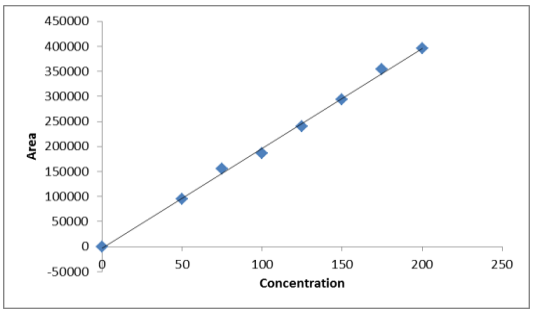


Figure 5: Calibration plot for Vandetanib

3.2 Precision

Precision was determined by using the method to assay a sample for a sufficient number of times to obtain statistically valid results. Precision of the method was performed as Intraday precision and Interday precision. The precision is then expressed as the relative standard deviation.

3.2.1 Intraday precision

The Intraday precision was studied by preparing and injecting six replicate standard solutions of Vandetanib (100 ppm) using the proposed method. The percent relative standard deviation (% RSD) was calculated for the peak areas and it was found to be 0.471%, which is well within the acceptance criteria of not more than 2.0%. Results of intraday system precision studies are shown in Table 3.

TABLE 3: Intra-day precision results

Sample	Conc. (in ppm)	Injection No.	Peak Area	% RSD
Vandetanib	100	1	186388.7	0.471
		2	184117.6	
		3	185300.5	
		4	184990.5	
		5	185043.5	
		6	186346.8	

3.2.2 Interday precision

The interday precision was studied by preparing and injecting six replicates of standard solutions of Vandetanib (100 ppm) on three different days over a period of one week. The percent relative standard

deviation (% RSD) was calculated and it was found to be 0.61%, which is well within the acceptance criteria of not more than 2.0%. Results of interday system precision studies are shown in Table 4.

TABLE 4: Inter-day precision results

Sample	Conc. (in ppm)	Injection No.	Peak Area	% RSD
Vandetanib	100	1	184080.1	0.61
		2	184614.3	
		3	184463.0	
		4	183561.0	
		5	185238.9	
		6	186789.4	

3.3 Selectivity

The selectivity of method was confirmed by comparing the chromatograms of blank, standard and tablet sample. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The results are shown in Table 5.

TABLE 5: Selectivity results

Name of the solution	Retention Time (in min)
Blank	No peak
Standard	3.326
Sample	3.315

3.4 Accuracy

The accuracy of the method was determined by calculating recovery of Vandetanib by taking 100 ppm as fixed concentration and comparing the true and observed areas. In order to establish accuracy 50% and 150% levels of this concentration was compared and the % recovery along with % RSD obtained for these are shown in Table 6. The accuracy was attained by performing three replicates of the above three

mentioned concentrations. The model sample of repeatability pertaining to the three concentrations is alone shown here.

TABLE 6: Accuracy results

S.No	Conc. (in ppm)	True Area	Observed area	Amount found	% Recovery	% RSD
1	50	95444	96378	50.489	100.978	
2	100	185676	186495	100.44	100.411	0.40
3	150	294114	297634	151.79	101.19	

3.5 Limit of detection and Limit of quantification

The sample was dissolved by using the mobile phase and injected until the peak disappeared. After 0.75 ppm dilution, peak was not observed clearly. So it confirms that 0.75 ppm is the Limit of Detection and Limit of Quantification was found to be 2.475 ppm.

3.6 Robustness

Robustness was carried out by varying three parameters from the optimized chromatographic conditions. The results are shown in Table 7.

TABLE 7: Robustness results

Parameter changed	Change	Area	% Recovery
Standard	-	185676.3	-
Mobile phase	acetonitrile : water : 0.1% OPA (85:13:2)	185791.8	100.06
Wavelength	245 nm	186343.0	100.35
pH	4.3	184231.5	99.22

3.7 Formulation

The validated method was applied for the assay of commercial tablets containing Vandetanib. The formulation tablets of Vandetanib were crushed to give finely powdered material. Powder equivalent to 10 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 100 ppm. An aliquot

of this solution was injected into HPLC system. Peak area of Vandetanib was measured and compared against the peak area of the standard solution. The proposed method was able to estimate Vandetanib in the tablet formulation with an accuracy of 99.708%. The effect of the excipients was absent. The results presented good agreement with the labeled content as shown in Table 8.

TABLE 8: Formulation results

Brand	Dose (mg)	Sample Conc.	Standard area	Sample area	Amount found	% assay
Caprelsa	100	100 ppm	185676	185134	99.708 ppm	99.708

4. CONCLUSION

The present investigation lead to the development of a simple, accurate and efficient RP-HPLC method for the determination of Vandetanib having a low retention time of 3.32 minutes within a short runtime of 5 minutes. Later using a mass detector the molecular ion was also detected and quantified at m/z 473.3. Detection was achieved on Gemini C18 5µm, (250×4.6 mm) column with multiple reaction monitoring of the ion transitions m/z 474.3 → 100.09. The present findings provide a scope for a simple, rapid, sensitive and reproducible RP-HPLC method for analysis of Vandetanib in both bulk and pharmaceutical dosage forms.

REFERENCES:

1. Wedge SR, Ogilvie DJ, Dukes M et.al,. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res., 2002; 62(16): 4645-55.
2. Ciardiello F, Caputo R, Damiano V, Troiani T, Vitagliano D, Carlomagno F, et.al., Clinical cancer research: An official journal of the American Association for Cancer Research.,2003; 9: 1546.
3. Joseph A. Zirrolli, Erica L. Bradshaw, Michael E. Long, Daniel L. Gustafson, Rapid and sensitive LC/MS/MS analysis of the novel tyrosine kinase inhibitor ZD6474 in mouse plasma and tissues, Journal of Pharmaceutical and Biomedical Analysis, 2005; 39(3–4): 705–711.
4. Bai F, Johnson J, Wang F, Yang L, Broniscer A, Stewart CF, Determination of vandetanib in human plasma and cerebrospinal fluid by liquid chromatography electrospray ionization tandem mass spectrometry, J Chromatogr B Analyt Technol Biomed Life Sci.,2011; 879(25): 2561- 2566.

5. Minocha M, Khurana V, Mitra AK, Determination of pazopanib (GW- 786034) in mouse plasma and brain tissue by liquid chromatography- tandem mass spectrometry, J Chromatogr B Analyt Technol Biomed Life Sci., 2012; 901: 85-92.
6. Andriamanana I, Gana I, Duretz B, Hulin A, Simultaneous analysis of anticancer agents bortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS. Journal of Chromatography B. 2013 May 1;926:83-91.
7. Lin H, Cui D, Cao Z, Bu Q, Xu Y, Zhao Y, Validation of a high-performance liquid chromatographic ultraviolet detection method for the quantification of vandetanib in rat plasma and its application to pharmacokinetic studies. J Can Res Ther 2014;10:84-8.
8. Amer SM, Kadi AA, Darwish HW, Attwa MW., Liquid chromatography tandem mass spectrometry method for the quantification of vandetanib in human plasma and rat liver microsomes matrices: metabolic stability investigation.Chem Cent J. 2017;11(1):45.
9. Kadi AA, Abdelhameed AS, Darwish HW, Attwa MW, Bakheit AH. Liquid chromatographic-tandem mass spectrometric assay for simultaneous quantitation of tofacitinib, cabozantinib and afatinib in human plasma and urine. Trop J Pharm Res 2016; 15(12):2683-2692.
10. Ali S. Abdelhameed et.al., Development of novel univariate and multivariate validated chemometric methods for the analysis of dasatinib, sorafenib, and vandetanib in pure form, dosage forms and biological fluids, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2022; 264: 120336.
11. Ali S. Abdelhameed et.al., Simple and efficient spectroscopic-based univariate sequential methods for simultaneous quantitative analysis of vandetanib, dasatinib, and sorafenib in pharmaceutical preparations and biological fluids, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2021; 260: 119987.
12. Hany W.Darwish et.al., Development of novel response surface methodology-assisted micellar enhanced synchronous spectrofluorimetric method for determination of vandetanib in tablets, human plasma and urine, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2019; 213: 272-280.
13. Darwish HW, Bakheit AH. A new spectrofluorimetric assay method for vandetanib in tablets, plasma

and urine. Tropical Journal of Pharmaceutical Research. 2016;15(10):2219-25.

14. Khandare B, Dudhe PB, Upasani S, Dhoke M. Spectrophotometric Determination of Vandetanib in Bulk by Area Under Curve and First Order Derivative Methods. International Journal of Pharm Tech Research, 2019: 12(2): 103-110.