Original Article

Development of Validated Stability Indicating Method for Estimation of Vandetanib and Characterization of its Degradants by LC-ESI-MS

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ABSTRACT

Aim: In the current study, stability indicating high performance liquid chromatography method (RP-HPLC) was developed and validated for the determination of Vandetanib, also its major degradants were identified and characterized by Liquid Chromatography-Tandem Mass spectrophotometric method (LC-ESI-MS). **Methods and Materials:** This method was developed on Nucleosil 100-5, C_{18} (250 × 4.6 mm, 5μm) column by using Methanol: Ammonium acetate buffer as Mobile phase in the ratio, 90:10 v/v, having flow rate of 1 ml/min. The estimation was carried out at 249 nm. Further Vandetanib was subjected to various stress condition like acidic, alkali, oxidative, thermal and photolytic degradation. The degradation pathways for major degradants were idenitifed. **Results:** The method was developed and validated for linearity, robustness, accuracy, precision, linear regression analysis data which indicates the good linear relationship, correlation coefficient was found 0.992 in the concentration range of 1-10 μg/ml. In the stress results, the degradation of drug in alkaline, as well as acidic medium showed significantly. The product degradation was characterized by the LC-MS technique. **Conclusion:** The developed method was found to be rapid, sensitive, accurate, precise, and robust for the analysis of Vandetanib by which routine analysis of drugs can be done.

Key words: Mass Spectroscopy (MS), Vandetanib, High-Performance Liquid Chromatography (HPLC), Validation, Stress degradation.

INTRODUCTION

Vandetanib is chemically *N*- (4 – bromo – 2 - flurophenyl) – 6 – methoxy – 7 - ((1-methylpiperidin-4-yl) methoxy) quinazolin – 4 - amine. It is used in the treatment of certain tumours of the thyroid gland. It blocks the activity of kinase and act as kinase inhibitor of a number of cell receptors, including the epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and the RET-tyrosinekinase.[1] Literature survey shows that methods including UV-Vis spectoscropy,[2] Spectrofluorimetry,[3] bioanalytical HPLC,[4] bioanalytical LC-MS[5,6] have been published for the estimation of Vandetanib. This work describes development of simple, reliable HPLC system and validation according to ICH guidelines for the determination of Vandetanib in bulk form as well as identification and characterization of major degradants by liquid chromatographic–tandem mass spectrometric method (LC-ESI-MS). Figure 1 presents structure of vandetanib.

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MATERIALS AND METHODS

Reagents and chemicals

Methanol (HPLC grade), Acetonitrile (HPLC grade) were purchased from MERCK, Mumbai. Hydrochloric acid (HCl), Hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH),

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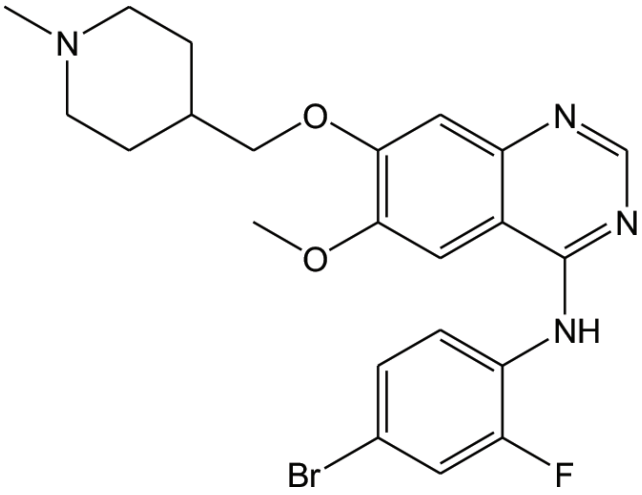

Figure 1: Chemical structure of Vandetanib.

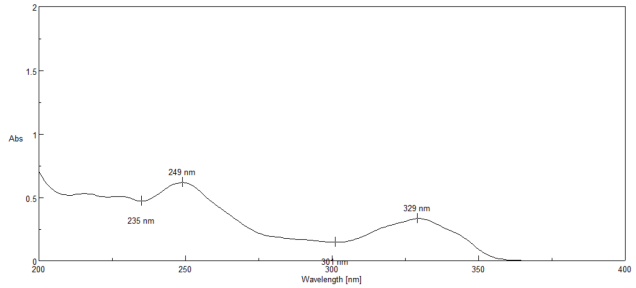

Figure 2: UV Spectra of Vandetanib (10 µg/ ml).

Ammonium acetate AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai. HPLC water generated through PURELAB UHQ-II HPLC water purification system was used.

Preparation of standard stock and buff

The standard stock solution of 1000 µg/ml was prepared by dissolving 10 mg of Vandetanib drug in 10 ml of Methanol. From the above solution 1 ml was pipette out and diluted to 10 ml with mobile phase to get the concentration of 100 µg/ml of Vandetanib. Further dilutions were made in methanol. Buffer was prepared by dissolving 77.1 g of Ammonium acetate in water, 57 ml of glacial acetic acid and diluted with water to 1000 ml.

Selection of wavelength

Further dilutions were made from the standard stock solution (1000µg/ml) using methanol and scanned using visible range of 200- 400 nm using UV spectrophotometer, spectra was obtained and maximum wavelength was found to be at 249 nm. A UV spectrum of Vandetanib is given in Figure 2.

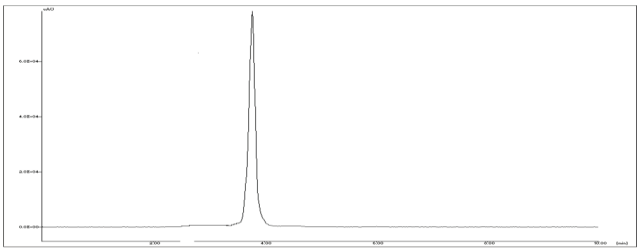

Figure 3: Chromatogram of Standard Vandetanib (10 µg/ ml).

Chromatographic conditions

HPLC system used was JASCO system equipped with model PU 2080 Plus pump, Rheodyne sample injection port (20µl), JASCO MD2010 Plus detector and Borwin chromatography software (version 1.5). A chromatographic column used was Nucleosil 100-5 C_{18} (250 × 4.6 mm, 5µm) operated at flow rate of 1.0 ml/min using Methanol: Ammonium acetate Buffer in the ratio of 90:10 v/v as mobile phase and wavelength detection at 249 nm. Figure 3 represents standard chromatogram of 10 ppm of Vandetanib.

Stress Degradation Studies of Bulk Drug

Stress degradation studies were performed under certain conditions like base, acid, neutral hydrolysis, oxidation, Photolysis and dry heat. Two samples were prepared (Blank and of Vandetanib RS) for each study. The blank and the drug solution were subjected to stress in the same manner. Photolytic degradation and dry heat were performed in solid state. A summary of stress degradation study of drug Vandetanib is given in Table 1.

Alkaline hydrolysis

Standard solution of Vandetanib (1000 µg/ml), 1 ml was mixed with 1 ml of 1 N NaOH and the solution was kept for overnight in dark place. Solution was neutralized with 1N HCl and made volume to 10 ml. 1 ml of resulting solution was diluted to 10 ml with mobile phase (10 µg/ml) and it was injected under optimized chromatographic condition. After alkaline degradation, Vandetanib showed one peak of degradation product at R_t 5.91. The percent recovery of Vandetanib was 67.52 % (Figure 4)

Acidic hydrolysis

Standard solution of Vandetanib (1000 µg/ml), 1 ml was added to 1 ml of methanolic 1 N HCl and the resulting solution was kept for overnight in dark place. Solution was neutralized with 1N NaOH, Volume made to 10 ml and the resulting solution, 1 ml was

Table 1: Summary of stress degradation study of Vandetanib.

Sr. No.	Stress Degradation Condition	% Recovery	R.T. of degraded products
1	Base (1 N NaOH, kept for overnight)	67.52	VD1 -5.91
2	Acid (1 N HCl, Kept for overnight)	85.73	VD1 -5.92
3	H_2O_2, 30% (Kept for overnight)	87.52	
4	Dry Heat (80°C for 6 hr)	98.95	
5	Photo stability [UV, 200 watt hrs/square meter Florescence, 1.2 million Lux. Hrs]	98.53	

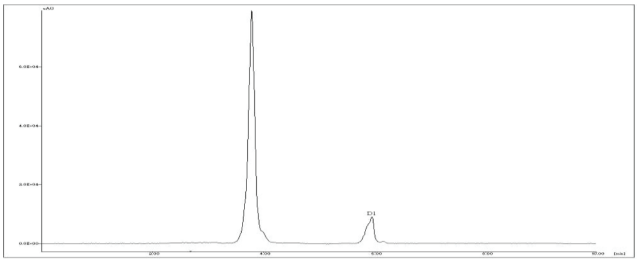

Figure 4: Chromatogram of Vandetanib (10 µg/ ml) after alkaline hydrolysis.

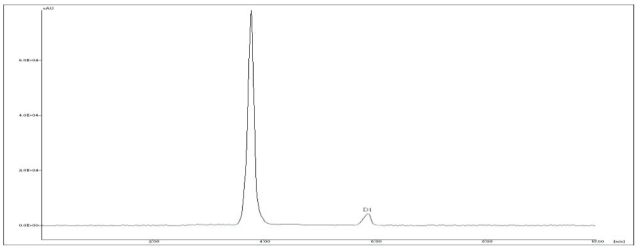

Figure 5: Chromatogram of Vandetanib (10 µg/ ml) after acid degradation.

diluted to 10 ml with mobile phase (10 µg/ ml) and then it was injected under optimized chromatographic condition. After acid hydrolysis, Vandetanib showed one peak of degraded product (at RT 5.92) with 85.73 % recovery (Figure 5).

Oxidative Degradation

Standard solution of Vandetanib (1000 µg/ml), 1 ml was added to 30 % solution of Hydrogen peroxide (1 ml) and the solution was allowed to stand for overnight in dark place. Volume made to 10 ml and the resulting solution, 1 ml was diluted to 10 ml with mobile phase (10 µg/ ml) and then it was injected under optimized chromatographic condition. In the

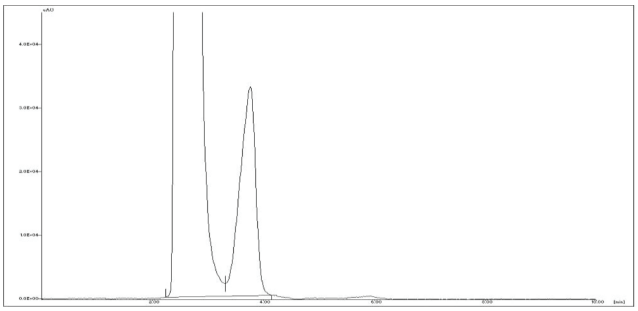

Figure 6: Chromatogram of Vandetanib (10 µg/ ml) after oxidation.

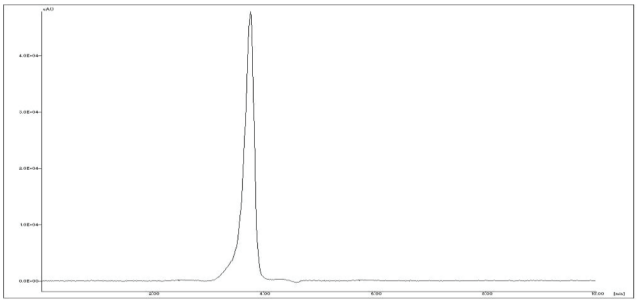

Figure 7: Chromatogram of Vandetanib (10 µg/ ml) after exposing to dry heat.

oxidative condition, percent recovery obtained for Vandetanib was 87.52 % with no peak of degradation observed in chromatograph (Figure 6).

Degradation under dry heat

In the dry heat studies, drug sample was kept in oven (80°C) for 6 hr. After specified period of time, sample was withdrawn and solution was prepared having concentration of 100 µg/ ml same as standard solution preparation. The resulting solution, 1 ml was further diluted to 10 ml with mobile phase (10 µg/ ml) and it was injected under optimized chromatographic condition. In the dry heat degradation condition, percent recovery obtained for Vandetanib was 98.95 % with no peak of degradation product (Figure 7).

Photo-degradation studies

Photolytic studies were performed by the exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hr. The sample withdrawn after exposure and processed as per standard solution preparation procedure mentioned under 6.5 to get 100 µg/ ml as solution concentration. The 1 ml of resulting solution was diluted with mobile phase to 10 ml (10 µg/ ml) and then was injected under optimized chromatographic condition.[7] The photo degradation

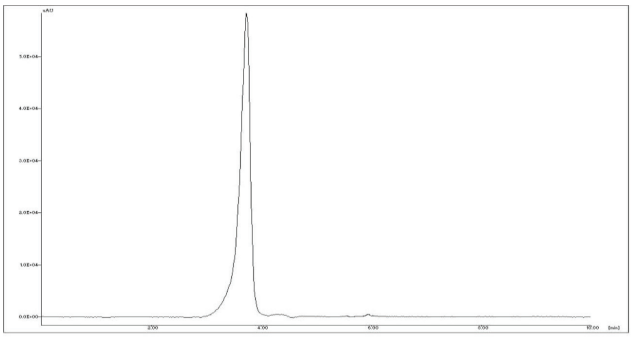

Figure 8: Chromatogram of Vandetanib (10 µg/ ml) after photo degradation.

study for UV light and Fluorescence light, Vandetanib showed no peak of degraded product with 98.53 % recovery (Figure 8).

Identification, Characterization and Prediction of Degradation Product by LC–MS

Forced degradation studies were performed as per ICH guidelines Q1A (R2). All solutions and stressed solid samples were fortified, wrapped with aluminum foil, refrigerated at -4°C for analysis. The LC-MS study was performed for standard drug as well as for forced degradation samples of drug for acid, alkali and oxidation under which degradation was observed during development of stability indicating assay method.

Successful separation of one degradation product under acidic as well as alkali condition was observed by high performance liquid chromatography (HPLC) using Zorbax Eclipse XDB-C_{18} (150 mm× 3.0 mm, 3.55 µm) analytical column. It was identified and characterized by liquid chromatography-electrospray ionization mass spectrometry (LC–ESI/MS) with accurate mass measurements up to four decimal.

Mass Spectroscopy Conditions

Mass Spec parameters were optimized for drugs to get highest intensity while tuning for scan to identify the [M + H]. Quantification was achieved with MS detection in positive ion mode for the drugs and its degrading products. Agilent LC-MS Q – TOF (6200 series TOF/6500 series) (5301 Stevens Creek Blvd, Santa Clara, CA 95051, United States) equipped with a dual AJS ESI with improved sensitivity [AJS – ESI: Agilent Jet Stream Electro spray Ionizer] and Q-TOF B.05.01 software version. The Min Range and Max Range in acquisition mode was set to 60 and 1000, respectively with scan rate of 2 spectra /sec.

Chromatograph of Vandetanib subjected to Acidic stress is given in Figure 9. LC-MS Spectrum of Vandetanib

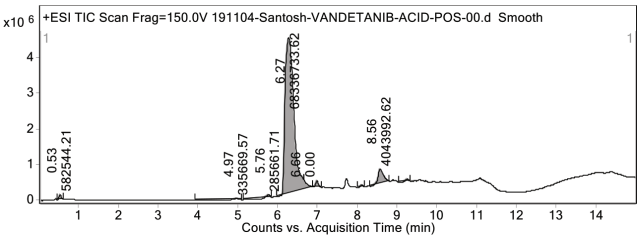

Figure 9: Chromatograph of Vandetanib subjected to Acidic stress.

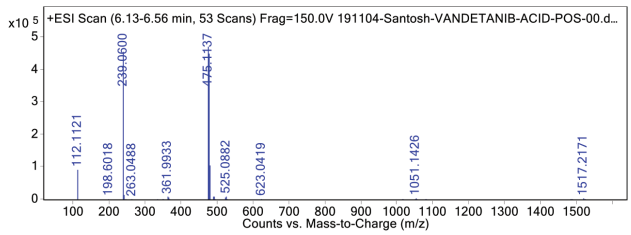

Figure 10: LC-MS Spectrum of Vandetanib from acidic stress sample (Retention Time – 6.27 min).

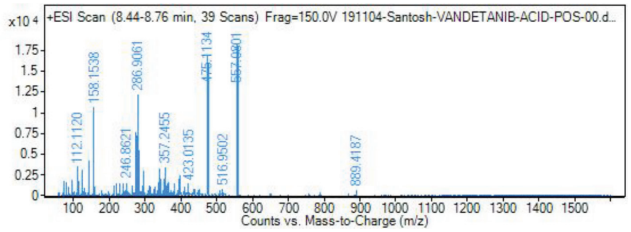

Figure 11: LC -MS Spectrum of VD1 of Vandetanib acid stress sample (Retention Time – 8.56 min).

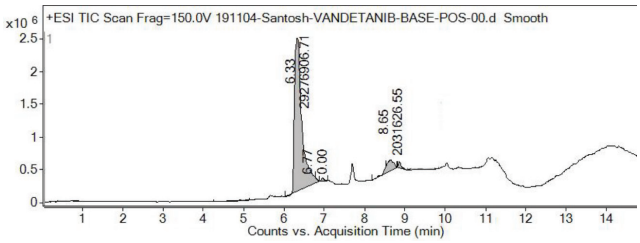

Figure 12: Chromatograph of Vandetanib subjected to Alkali stress.

and its degradant VD1 from acidic stress sample is given in Figure 10 and Figure 11. Chromatograph of Vandetanib subjected to Alkali stress is given in Figure 12. LC-MS Spectrum of Vandetanib and its degradant VD1 from acidic stress sample is given in Figure 13 and Figure 14. Probable degradation pathway of Vandetanib under Acid/Alkali stress sample is presented in Figure 15.

Analytical Method Validation

The method that was developed, validated as per ICH Q2 (R1) guidelines.[8]

Specificity

The method specificity was determined by peak purity profiling studies. The values of peak purity were found to be more than 998, indicating that there is no disturbance of any other degradation product peak, matrix and impurity.

Range and Linearity

Further dilutions were made with mobile phase from the Vandetanib standard stock solution (100 µg/ ml) to make solutions of six different concentrations. These six solutions per concentration were injected. The linearity i.e. relationship between peak area v/s concentration was determined over the concentration range of 1-10 µg/ml. The results are shown in Table 2. The graph was plotted against the peak area and corresponding concentrations for obtaining the calibration curve. The results found linear with regression equation of Y=85361X+46194 and correlation coefficient of 0.992.

Precision

The precision studies were carried out for the developed method by performing Inter-day and Intra-day variation studies. Intraday studies were performed by analyzing the 3 replicates of 3 different concentrations (2, 4, 5 µg/ml) of Vandetanib in a day and percentage RSD was calculated. Inter day studies were performed by analyzing

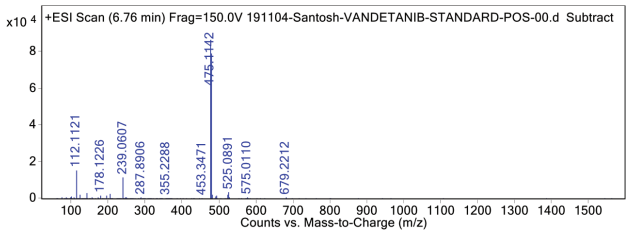

Figure 13: LC-MS Spectrum of Vandetanib from alkali stress sample (Retention Time – 6.33 min).

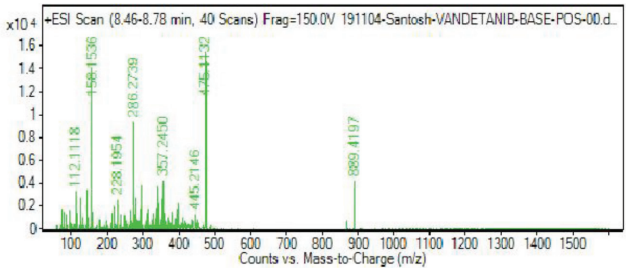

Figure 14: LC -MS Spectrum of VD1 of Vandetanib alkali stress sample (Retention Time – 8.65 min).

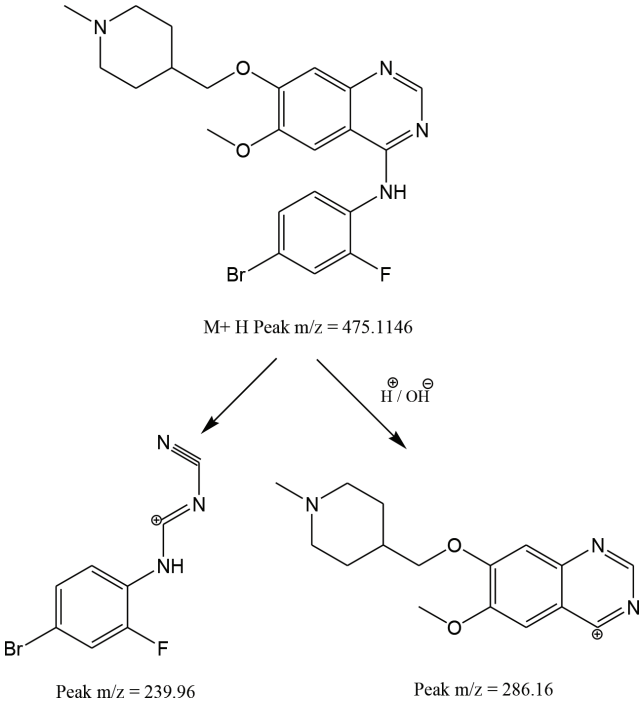

Figure 15: Probable degradation pathway of Vandetanib under Acid/Alkali stress sample.

Table 2: Linearity study of Vandetanib.

Replicates	Concentrations of Vandetanib					
	1 µg/ml	2 µg/ml	3 µg/ml	4 µg/ml	5 µg/ml	10µg/ml
	Peak Area					
1	124015.4	212342.4	294585	368249	529420.8	888174
2	127300.5	221236.4	289042	370148	516344.1	890665
3	130291.3	220485	279026	379630	516875.4	879632
4	126550.8	216294.5	291468	370098	523445.7	874635
5	130113.5	215782.5	293213	359237	517796.4	905476
6	129052.7	215484.8	284350	366922	522145.5	887624
Mean	127887.4	216937.59	288614	369047.2	521004.6	887700.9
Std. Dev.	2417.24	3347.76	5920.26	6568.425	5042.95	10590.56
%RSD	1.89	1.543	2.05	1.779	0.967	1.19

Table 3: Intra-day precision study Vandetanib.								
Theorotical Conc (mcg/ml)	Area	Slope	Intercept	Practical Conc	% assay	Avg*	SD	RSD
2	215168.976	85361	46194	1.980	98.977			
2	218398.764	85361	46194	2.017	100.869	100.245	1.098	1.095
2	218433.365	85361	46194	2.018	100.889			
4	389527.102	85361	46194	4.022	100.553			
4	384547.19	85361	46194	3.964	99.095	99.728	0.748	0.750
4	386051.63	85361	46194	3.981	99.535			
5	475045.778	85361	46194	5.024	100.480			
5	474626.91	85361	46194	5.019	100.381	100.149	0.489	0.489
5	471236.962	85361	46194	4.979	99.587			

*Average of three determinations

Table 4: Inter-day precision of Vandetanib.								
Theo. Conc (mcg/ml)	Area	Slope	Intercept	Practical Conc	% assay	Avg*	SD	RSD
2	217342.4	85361	46194	2.005	100.250			
2	216236.4	85361	46194	1.992	99.602	99.866	0.340	0.340
2	216485.0	85361	46194	1.995	99.748			
4	390699.2	85361	46194	4.036	100.897			
4	386868.4	85361	46194	3.991	99.775	100.373	0.565	0.563
4	389164.95	85361	46194	4.018	100.447			
5	469877.13	85361	46194	4.963	99.269			
5	470860.03	85361	46194	4.975	99.499	99.599	0.391	0.392
5	473129.48	85361	46194	5.002	100.031			

*Average of three determinations

the 3 replicates of different concentrations of Vandetanib on three consecutive days and percentage RSD were calculated. The results for Inter-day and Intra day variation studies are shown in Table 3 and Table 4.

LOD (Limit of Detection) and LOQ (Limit of Quantification)

Limit of detection and Limit of Quantitation was calculated from the linearity data by using the formula LOQ = 10 σ / S and LOD = 3.3 σ / S where, σ = SD of the Y-intercept and S = Slope of the calibration curve. The LOD and LOQ were found to be 0.146 and 0.443. The formulation analysis of Vandetanib is shown in Table 5.

Table 5: Results of formulation analysis of Vandetanib (Assay).			
Sr. No.	Area	Concentration (µg/ml)	% Recovery
01	216685.6	1.997	99.865
02	217752.4	2.010	100.490
03	219831.9	2.034	101.708
04	218046.5	2.013	100.662
05	214913.3	1.977	98.827
06	215739.4	1.986	99.311
Mean	217161.517	2.003	100.144
S.D	1764.465	0.021	1.034
% RSD	0.813	1.032	1.032

Assay

Preparation of Blend (Synthetic mixture)

Assay of Vandetanib was done by spike blend method due to unavailability of its marketed preparation. 300 mg of Vandetanib was mixed with equal amounts (100 mg) of starch and lactose to make 500 mg of spike blend. The contents of spike blend were properly mixed. Assay was performed on blend of bulk drug with excipients. A quantity of synthetic mixture (Blend) and equal amount of 10 mg of Vandetanib was mixed with 5 ml

Table 6: Recovery study of Vandetanib.

Level	Sample Conc. (µg/ml)	Std Conc. (µg/ml)	Area	Amount Recovered	% recovery (Mean ±%RSD)
50 %	2	1	294939.4	2.914	100.427 ± 0.920
			296025.3	2.927	
			296473.7	2.932	
100 %	2	2	378524.2	3.893	99.199 ± 0.498
			379621.8	3.906	
			380180.9	3.913	
150 %	2	3	461790.6	4.869	99.982 ±1.609
			466593.8	4.925	
			469988.2	4.965	

methanol in a 10 ml volumetric flask. The resulting mixture was then ultra sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and volume was made up with the methanol to make the concentration of1000 µg/ml. Final solution of concentration 2 µg/ml was made in mobile phase. Procedure was repeated for six times. Percentage assay was determined by extrapolation of peak area from linearity equation.

Accuracy

Recovery studies were performed for checking the accuracy of the developed method. For this purpose the standard drug was added to the sample at three different levels 50, 100 and 150 %. The concentration of sample solution, 2 µg/ ml was chosen as basic concentration. These solutions were injected three times in stabilized chromatographic conditions to obtain the chromatograms. The Vandetanib drug concentrations were calculated by using linearity equation. The results are shown in Table 6.

Robustness

Robustness of the developed method was checked by altering the mobile phase composition, flow rate (± 0.05 ml/min), wavelength detection (± 1 nm) under certain conditions and the effect on the area was noted. The robustness of the method was performed by alterations done in the analytical parameters, but the peak area remained unaffected that indicates that the method is robust.

CONCLUSION

A simple, rapid, precise, accurate and sensitive high performance liquid chromatographic (HPLC) method was developed and validated for the analysis of Vandetanib in bulk and tablet dosage form. Separation was done on Nucleosil 100-5 C_{18} (250 × 4.6 mm, 5µm) column. Methanol: Ammonium acetate Buffer was used as mobile phase in the ratio of 90:10 v/v and at flow rate of 1 ml/min and maximum wavelength was detected at 249 nm. The retention time (RT) of drug Vandetanib was found to be 3.717 ± 0.034 min. The developed method was validated for linearity, range, accuracy, robustness, method precision (intraday and inter day). The linear regression analysis data indicated that a good linear relationship showed in the concentration range of 1-10 µg/ml. The correlation coefficient (R^2) was found to be 0.992. The % assay was found to be 100.144± 1.032. The developed method can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **ICH:** International Conference on Harmonization; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **HCl:** Hydrochloric acid; **NaOH:** Sodium Hydroxide; **H_2O_2:** Hydrogen Peroxide; **RSD:** Relative Standard Deviation; **SD:** Standard Deviation.

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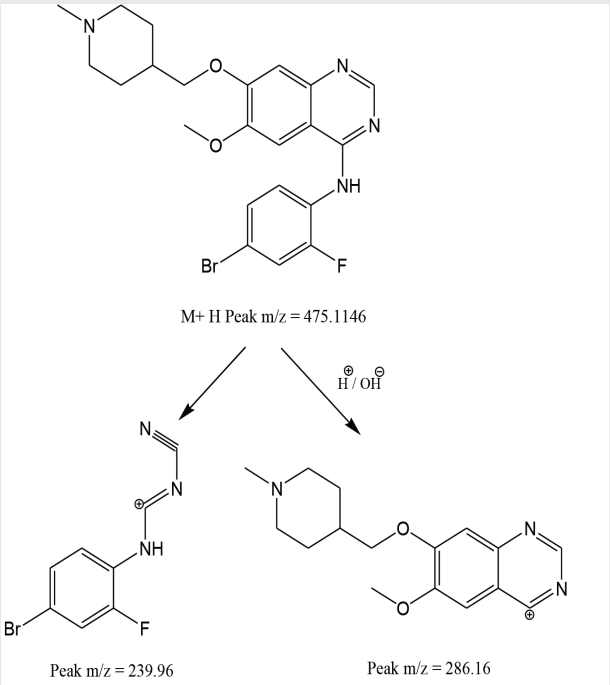
PICTORIAL ABSTRACT



Identification and Structural Characterization of Degradation Products of Vandetanib

SUMMARY

The objective of the study was to develop and validate rapid and accurate stability indicating High Performance Liquid Chromatography method for the estimation of Vandetanib and to identify and characterize degradants by LC-ESI-MS. Separation was carried on JASCO system with Nucleosil 100-5 C_{18} (250 x 4.6 mm, 5μm) column. Methanol: Ammonium acetate Buffer was used as mobile phase in the ratio of 90:10 v/v and at flow rate of 1 ml/min and maximum wavelength was detected at 249 nm. The retention time (R_t) of drug Vandetanib was found to be 3.717 ± 0.034 min. The method that was developed validated as per ICH guidelines. The linear regression analysis data indicated that a good linear relationship showed in the concentration range of 1-10 μg/ml. The % assay was found to be 100.144 ± 1.032. The developed method is stability indicating and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

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