RESEARCH ARTICLE

Development and Validation of Stability Indicating Liquid Chromatographic Method for Vandetanib: Application to Degradation Studies by LC-MS

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ABSTRACT

Objective: The central goal of this research was to formulate and validate a stability-demonstrating assay approach using reverse-phase high-performance liquid chromatography (RP-HPLC) for the assessment of vandetanib, its primary degradation products, and their respective degradation pathways, which were identified and characterized using liquid chromatography-electrophore spray ionization-mass spectrometry (LC-ESI-MS).

Methods: The method was designed and developed by employing a Grace C18 column (250 x 4.6 mm x4.0 µm) and deploying a mixture of methanol and 0.1% formic acid in H_2O (in a proportion of 90:10, v/v) as the mobile phase, with a flow rate maintained at 1-mL/min. The analytes were detected at a wavelength of 249 nm. Additionally, vandetanib was subjected to various stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic degradation, to unearth the degradation paths for the principal degradation products.

Results: The approach was meticulously formulated and validated to ensure robustness, linearity, precision, accuracy, and linear regression analysis data. A high correlation coefficient of 0.9985 was obtained in the 2 to 12 µg/mL concentration range, demonstrating a robust linear relationship. Moreover, the limit of detection (LoD) and the limit of quantification (LoQ) were found to be 0.166 and 0.503 µg/mL, respectively. In the stress degradation studies, we noticed that vandetanib degraded in alkaline and acidic mediums. The liquid chromatography-mass spectrometry (LC-MS) technique was used to characterize the degradation products.

Conclusion: The devised method demonstrated itself to be quick, sensitive, precise, accurate, and robust for the vandetanib analysis, thereby facilitating its routine testing.

Keywords: Vandetanib, LC-ESI/MS, Stress degradation, Validation.

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INTRODUCTION

Vandetanib, sold as caprelsa, is a potent anti-cancer drug used in the therapy of specific thyroid gland tumors. It is also being studied in the treatment of other types of cancers. It acts by inhibiting specific proteins that facilitate the growth of cancer cells. Besides, it is under exploration for its potential application in managing other cancer types. It finds its specific application in treating medullary thyroid cancer (MTC) that is either locally advanced, cannot be surgically removed, or has metastasized to other parts of the body. AstraZeneca developed the drug, and it belongs to the broad family of 4-aniliniquinazoline drug molecules that mimic adenosine and bind at the intracellular receptor site of tyrosine kinase. Vandetanib is particularly effective in extending progression-free survival and stabilizing disease in patients with MTC. • Vandetanib (Figure 1) is essentially a multi-kinase inhibitor used to treat advanced or metastatic MTC.[1] Vandetanib is an orally bioavailable 4-anilinouinazoline.

Furthermore, its versatility extends beyond its role as a single-agent therapy for MTC. Clinical investigations have begun exploring its potential in combination with other anti-cancer agents, aiming to enhance treatment outcomes and overcome drug resistance mechanisms observed in some patients. Preliminary data from pre-clinical and early-

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phase clinical trials show promising synergistic effects when vandetanib is administered alongside traditional chemotherapy or targeted therapies, suggesting a promising avenue for personalized cancer treatment regimens.

In addition to its direct anti-tumor effects, vandetanib exhibits a favorable safety profile, contributing to its suitability for long-term treatment strategies. While adverse events such as diarrhea, rash, and hypertension have been reported, they are generally manageable with appropriate supportive care and dose adjustments. Moreover, ongoing pharmacovigilance efforts continue to refine our understanding of vandetanib's safety profile and inform clinical practice guidelines, ensuring its optimal utilization in the ever-evolving landscape of cancer therapy.

IUPAC name

N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl) methoxy] quinazolin-4-amine.

Class

Antineoplastics, Tyrosine kinase blocker.

Molecular Formula

C22H24BrFN4O2

Molecular Weight

475.4

Vandetanib displays pH-dependent aqueous solubility and is categorized as having low solubility.

Mechanism

Vandetanib operates as an inhibitor of a kinase, and P-glycoprotein inhibitor and organic cation transporter-2 inhibitor, explicitly focusing on cellular receptors like the epidermal growth factor receptor, vascular endothelial growth factor receptor, and RET-tyrosine kinase.[2] It effectively halts vascular endothelial growth factor (VEGF)-stimulated processes like endothelial cell movement, propagation, survival, and angiogenesis.[3] This inhibition ultimately leads to decreased tumor growth and improved clinical outcomes in patients with cancers such as MTC and non-small cell lung cancer.

Overall, vandetanib's mechanism of action involves targeting multiple signaling pathways implicated in cancer growth and progression, including epidermal growth factor receptor (EGFR), VEGFR and RET pathways.

Solubility

Methanol, di-methyl sulfoxide, and di-methyl formamide. Sparingly soluble in aqueous buffers.
pKa: 5.2

MATERIALS AND METHODS

Chemicals and Reagent Procurement

The vandetanib drug used for this study was generously provided as a gift sample from AstraZeneca. A variety of chemical substances were utilized, including hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide

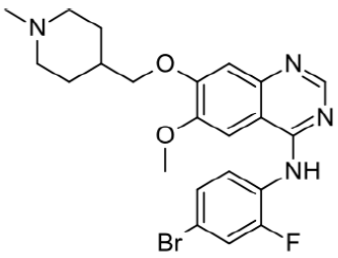

Figure 1: Molecular makeup of vandetanib

-30% w/v (H_2O_2), formic acid (CH_2O_2) of analytical reagent grade. Methanol and water of high-performance liquid chromatography (HPLC) grade were also used. Loba Chemie Pvt. Ltd, Mumbai, was the source of all these purchased chemicals.

Instruments and Make and Model

High profile liquid chromatography- JASCO(PU-2080) Borwin Chromatography Software (Ver-1.5)

UV-visible spectrophotometer- JASCO model V-730
Balance- Shimatzu, ATX-224
Ultrasonicator- Biomedica
Water purification system- Lab link
Photostability chamber-Neutronic-NEC103RSPI.
Hot air oven-Biomedica

Formulation of Primary Stock Solution

The main stock mixture of Vandetanib was created by mixing 10 mg of it in ten mL of methanol, yielding a 1000 µg/mL concentration. To get a 100 µg/mL vandetanib concentration, 1-mL from this primary stock solution was further watered down to 10 mL using the mobile phase. Additional dilution processes were carried out using methanol.

Identification of Detection Wavelength

More dilutions were made from the primary stock solution using methanol, and their absorbance was measured within the spectral region of 200 to 400 nm to obtain the absorption profile. It was noted that vandetanib showed significant light absorbance at 249 nm (Figure 2).

Identification of Mobile Phase Composition and Chromatographic Conditions

An investigation was conducted on the vandetanib standard working solution (10 µg/mL). At the outset, experiments were

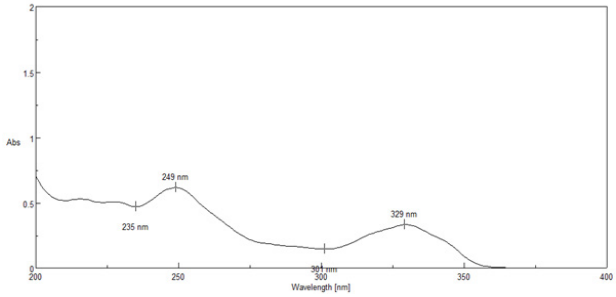

Figure 2: UV spectra of vandetanib (10 µg/mL)

Table 1: Finalized chromatographic parameters

S No.	*Parameter*	*Specified conditions for analysis*
1.	Mobile Phase ratio	Methanol: 0.1% HCOOH 90:10 v/v
2.	Flow rate set	1 mL/min
3.	Detection wavelength measured	249 nm
4.	Sample injector utilized	20 µL
5.	Column utilized	Grace C_{18} (250 x 4.6 mm, 5 µm)
6.	Column temperature maintained	Room temperature

conducted with methanol and acetate buffers in varying ratios and buffers of differing pH levels to attain the necessary system suitability parameters. Following several trials, a methanol and formic acid mobile phase in the ratio of 90:10 was selected due to its satisfactory resolution and acceptable peak characteristics (Table 1).

RESULTS AND DISCUSSIONS

Formulation of Sample Solution (Assay)

About 20 tablets were accurately weighed and pulverized, and an amount corresponding to 10 mg of vandetanib was shifted into a volumetric flask of 10 mL containing methanol of 5 mL volume. Ultrasonic waves were applied to this flask for 10 minutes. The solution was passed through a Whatman filter paper 41 to remove undissolved particles. The solution's mixture was adjusted using methanol to achieve a 1000 µg/mL concentration. A mobile phase was utilized to dilute the solution to achieve a concentration of 4 µg/mL. This entire procedure was performed six times, and the assay was determined by extrapolating the peak response from the linearity equation.[4]

Drug Chromatogram and System Suitability Parameters

The vandetanib working standard solution (10 µg/mL) was introduced into the system. The retention intervals recorded for successive injections were proven to be 4.213 ± 0.067 minutes (Table 2). The corresponding chromatogram for the drug can be seen in the provided Figure 3.

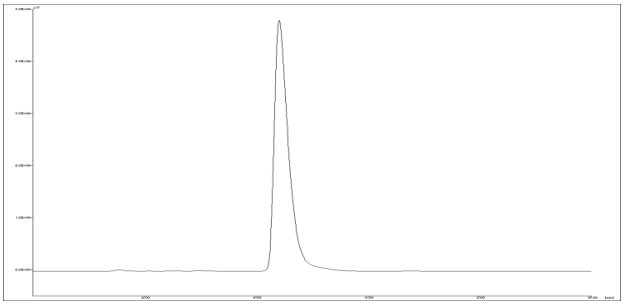

Figure 3: Chromatogram of standard vandetanib (10 µg/mL)

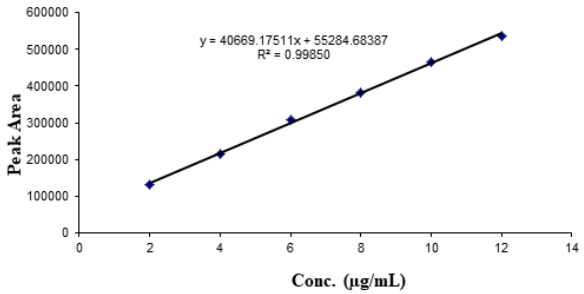

Figure 4: Linearity graph

Validation of Analytical Method

Linearity assessment

Additional dilutions were made from the primary stock mixture of vandetanib, which had a concentration of 1000 µg/mL, using the mobile phase to produce a solution encompassing six distinct concentrations. Each dilution was injected six times (Table 3). The linearity (Peak area and concentration relationship) was evaluated within the concentrated amount of 2 to 12 µg/mL (Figure 4).

Range

Vandetanib: 2 to 12 µg/mL

Precision

In the Intraday investigations, three replicas of three distinct concentrations (4, 8, 12 µg/mL) were assessed daily, and the

Table 2: System performance evaluation criteria

Name	*RT (Minute) ± RSD*	*Concentration (µg/mL)*	*Area (µV.sec)*	*Plates*	*Asymmetry*
Vandetanib	4.213 ± 0.067	10	466003.72	3464	1.13

Table 3: Linearity study of vandetanib

Conc (µg/mL)	*1*	*2*	*3*	*4*	*5*	*6*	*Avg*	*SD*	*RSD*
2	132164.76	131596.92	131149.20	133879.20	135018.98	131831.25	132606.72	1508.57	1.14
4	219684.92	216774.29	213595.20	210865.20	213231.20	212175.60	214387.73	3256.48	1.52
6	311110.80	306331.48	305698.12	307135.92	306316.92	317455.32	309008.09	4574.82	1.48
8	384984.60	383947.20	384553.26	381823.26	376423.32	378498.12	381704.96	3523.92	0.92
10	466003.72	464959.50	462407.40	463837.92	469347.06	464007.18	465093.80	2404.62	0.52
12	535893.54	535363.47	535462.20	541743.02	537864.60	535746.12	537012.16	2493.29	0.46

Table 4: Intraday precision study Vandetanib

Theo. conc (µg/mL)	*Area*	*Practical Conc*	*%Assay*	*Avg**	*SD*	*%RSD*
4	217335.30	3.99	99.62	100.35	0.64	0.64
4	219282.70	4.03	100.81			
4	218977.85	4.03	100.63			
8	381722.25	8.03	100.33	100.39	0.39	0.39
8	383251.05	8.06	100.80			
8	380707.60	8.002	100.02			
12	543006.10	11.99	99.94	99.79	0.38	0.38
12	540166.90	11.92	99.36			
12	543629.45	12.01	100.07			

Table 5: Inter-day precision study vandetanib

Theo. conc (µg/mL)	*Area*	*Practical Conc*	*%Assay*	*Avg**	*SD*	*%RSD*
4	219508.65	4.04	100.95	101.51	0.52	0.51
4	220556.43	4.06	101.60			
4	221167.63	4.08	101.97			
8	385539.47	8.12	101.51	101.75	0.24	0.23
8	387083.56	8.16	101.98			
8	386380.18	8.14	101.77			
12	539190.47	11.90	99.15	99.59	0.45	0.45
12	543575.31	12.01	100.05			
12	541148.79	11.95	99.56			

Table 6: Limit of detection and quantitation

Methodology	*Average slope*	*SD*	*LoD (µg/mL)*	*LoQ (µg/mL)*
S.D of y-intercept	40669.18	2046.84	0.166	0.503

Table 7: Drug specificity

Drug	*Purity tail*	*Purity front*
Vandetanib	998.16	998.58

%RSD was determined (Table 4). For the inter-day variation evaluations, three concentrations were assessed on three successive days, with % RSD calculated (Table 5).

Limit of quantitation and detection (Table 6)

In accordance with ICH recommendations, the LoD and LoQ were computed. A calibration graph was created by plotting the obtained area under each level's curve against the concentration. The slope and standard deviation were computed to find LOD and LOQ. It was found that the Limits of Quantitation (LOQ) and Detection (LOD) were, respectively, 0.166 µg/mL and 0.503 µg/mL.

Specificity (Table 7)

To be specific, a developed method's ability to separate and resolve vandetanib must be demonstrated. The method's specificity was assessed by measuring the peak purity and peak front. There was no interference from any other degradation product or impurity peak, as shown by peak purity values greater than 99.8.

Table 8: Assay results

S. No.	*Area*	*Concentration (µg/mL)*	*%Recovery*
01	217526.40	3.99	99.73
02	219055.20	4.03	100.67
03	218831.20	4.02	100.53
04	217861.44	4.00	99.93
05	219228.10	4.03	100.78
06	217417.20	3.99	99.67
Mean	218319.92	4.01	100.22
SD	810.14	0.02	0.50
%RSD	0.371	0.50	0.50

Assay

The sample solution was introduced into the system, and the resultant area under the curve was recorded. The linear regression equations determined the concentration and percentage recovery (Table 8).

Accuracy

The accuracy and recovery were evaluated by combining standard drug with the pre-analyzed three-level samples: 50, 100, and 150%. The selected level of concentration chosen

Table 9: Recovery study of vandetanib

Level (%)	*Concentration of sample (µg/mL)*	*Std concentration (µg/mL)*	*Area*	*Recovered amount*	*%Recovery*	*%Recovery (Mean ± %RSD)*
50	4	2	300317.50	6.03	100.42	99.79 ± 0.63
			297265.04	5.95	99.17	
			298782.32	5.99	99.79	
100	4	4	381281.04	8.02	100.20	100.32 ± 0.17
			381438.00	8.02	100.25	
			382313.88	8.04	100.52	
150	4	6	462353.76	10.01	100.09	99.97 ± 0.39
			463099.14	10.03	100.28	
			460085.76	9.95	99.54	

Table 10: Robustness study

Wavelength variation								
248 nm			*249 nm*			*250 nm*		
Area	*Change in TP*	*RT*	*Area*	*Change in TP*	*RT*	*Area*	*Change in TP*	*RT*
220806.95	3452	4.22	219332.75	3467	4.21	224119.35	3514	4.217
218450.05	3534	4.22	215883.85	3585	4.23	221921.7	3525	4.198
220106.25	3471	4.19	217576.45	3482	4.19	221225.55	3464	4.214
Avg: 219787.75	3485.67	4.21	217597.68	3511.33	4.21	222422.20	3501	4.21
SD:1210.30	42.92	0.01	1724.55	64.24	0.02	1510.43	32.51	0.01
RSD: 0.55	1.23	0.36	0.79	1.83	0.47	0.68	0.93	0.24
Flow rate								
0.95 mL			*1 mL*			*1.05 mL*		
Area	*Change in TP*	*RT*	*Area*	*Change in TP*	*RT*	*Area*	*Change in TP*	*RT*
219378.25	3478	4.256	219323.65	3461	4.22	188834.1	3463	4.13
215428.85	3562	4.261	215774.65	3576	4.22	188934.2	3548	4.14
217576.45	3489	4.251	216229.65	3480	4.21	184015.65	3468	4.12
217461.18	3509.67	4.26	217109.32	3505.67	4.22	187261.32	3493	4.13
1977.22	45.65	0.005	1931.12	61.65	0.006	2811.27	47.70	0.006
0.91	1.30	0.12	0.89	1.76	0.13	1.50	1.37	0.15
MP composition								
88:12			*90:10*			*92:08*		
Area	*Change in TP*	*RT*	*Area*	*Change in TP*	*RT*	*Area*	*Change in TP*	*RT*
219969.75	3471	4.25	208299	3463	4.21	210969.85	3507	4.18
215883.85	3562	4.26	205373.35	3555	4.22	214482.45	3578	4.19
217576.45	3489	4.27	212448.6	3479	4.23	213631.6	3465	4.18
217810.02	3507.33	4.26	208706.98	3499	4.22	213027.97	3516.67	4.18
2052.94	48.19	0.01	3555.23	49.15	0.01	1832.45	57.18	0.01
0.95	1.37	0.24	1.70	1.40	0.22	0.86	1.62	0.2

for the experiment was 4 µg/mL of the sample solution (Table 9). Triplicate injections of the standard vandetanib solutions were made under stable chromatographic conditions to obtain the corresponding chromatograms. The concentrations of vandetanib in the samples were determined with the help of an established linearity equation.

Robustness

The robustness of the method was evaluated by modifying certain parameters during the analysis. These modifications included alterations in the mobile phase's chemical makeup, adjustment of the detection wavelength (± 1 nm), and alterations to the flow rate by ± 0.05 mL/min (Table 10). Any

Table 11: Overview of validation standards

S. No.	*Parameter*	*Vandetanib*
1.	Linearity	y = 40669x + 55285 R^2 = 0.9985
2.	Range	2–12 µg/mL
3.	Assay (Mean ± %RSD)	100.221 ± 0.497
4.	Precision	(%RSD)
	Intra-day precision	0.379–0.642
	Inter-day precision	0.234–0.508
5.	Accuracy	%Recovery (Mean ± %RSD)
	50%	99.791 ± 0.627
	100%	100.320 ± 0.171
	150%	99.968 ± 0.386
6.	LoD	0.166 µg/mL
7.	LoQ	0.503 µg.mL
8.	Specificity	Specific
9.	Robustness	Robust

subsequent effect under the area under the curvature was noticed and recorded.[5-7]

Summary

Overview of validation standards is shown in Table 11.

Degradation analysis of the bulk drug under stress conditions

Stress-induced degradation trials were conducted under various conditions, including acidic, basic, and neutral hydrolysis, oxidative conditions, dry heat, and exposure to light (photolysis). Two sample types were prepared: One blank and one containing vandetanib. The blank sample was subjected to the same stress conditions as the vandetanib solution. Degradation due to dry heat and photolysis was tested in a solid state.[8]

- *Alkaline hydrolysis*

About 1-mL of vandetanib standard stock solution (1000 µg/mL) was combined with one milliliter of 2N NaOH and left undisturbed for 24 hours in a darkened space. The alkaline solution was then neutralized using 2N HCl. The volume of the solution was brought up to 10 mL, and the mobile phase was added to this one millimeter of neutralized solution to dilute it even more and achieve a 10 µg/mL concentration. This solution underwent chromatographic procedures that were optimized. The degraded vandetanib duct displayed two peaks at retention times (RT) of 2.916 and 3.758. The percent recovery for vandetanib was observed to be 57.42%.

- *Acidic hydrolysis*

A 1-mL of vandetanib's standard stock solution (1000 µg/mL) was combined with 1-mL of 2N HCl in methanol, and the mixture was stored in a dark environment for 24 hours. Post this period, the resultant mixture was neutralized with 2N NaOH. A 10 mL adjustment was made to the mixture's volume and a milliliter of this adjusted mixture was diluted even more with the mobile phase to yield a 10 µg/mL concentration. This mixture was then run under pre-determined chromatographic conditions. Vandetanib displayed a single peak for the degraded product at a retention time (RT) of 2.238, with a recovery rate of 81.49%. For chromatograms refer Figure 6A and 6B.

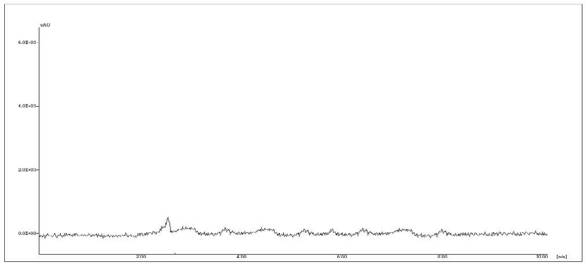

Figure 5A: Alkaline blank injections

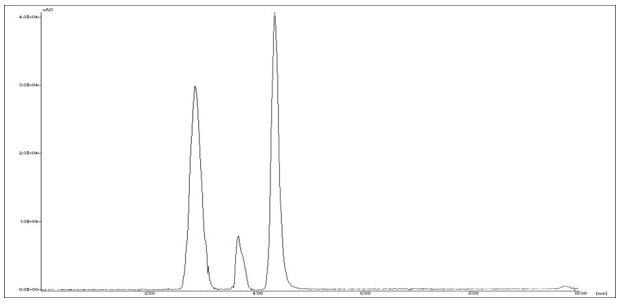

Figure 5B: Vandetanib (10 µg/mL) after alkaline degradation

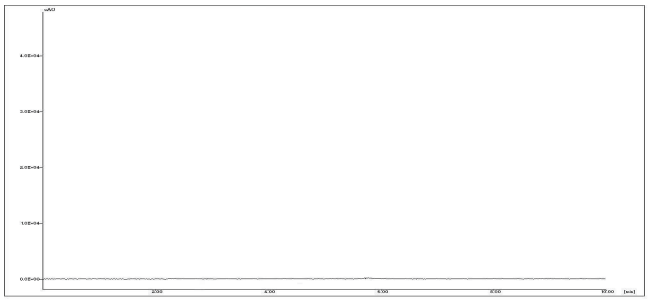

Figure 6A: Acid blank injections

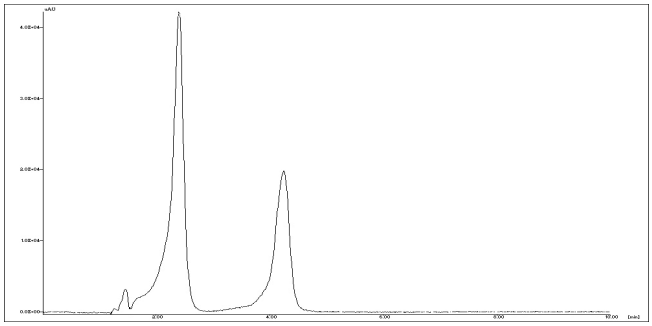

Figure 6B: Vandetanib (10 µg/mL) after acid degradation

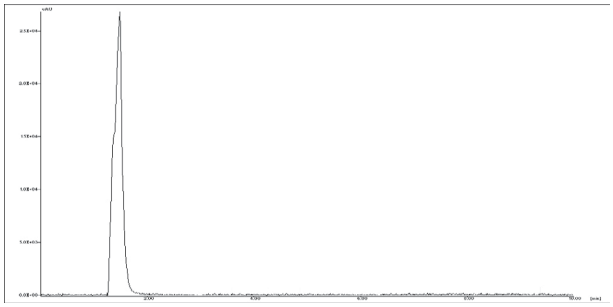

Figure 7A: Blank H_2O_2 injection

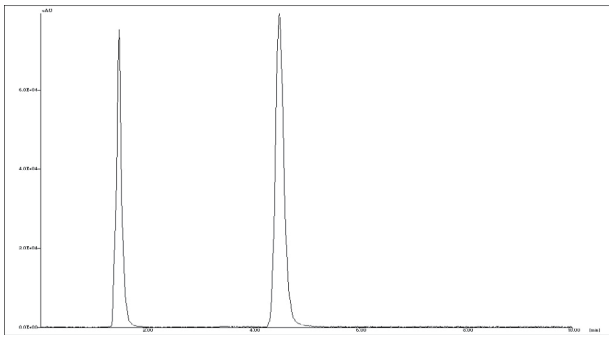

Figure 7B: Vandetanib (10 µg/mL) after oxidation

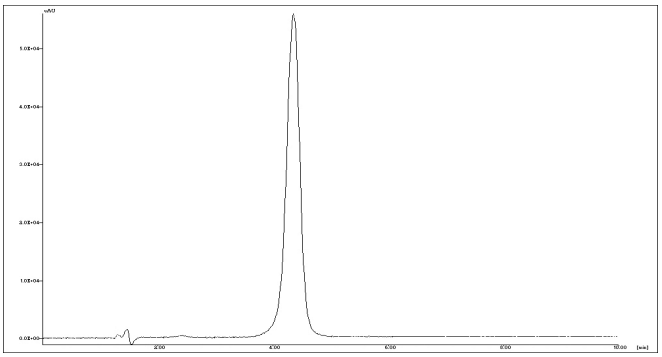

Figure 8: Chromatogram of vandetanib (10 µg/mL) after exposure to dry heat

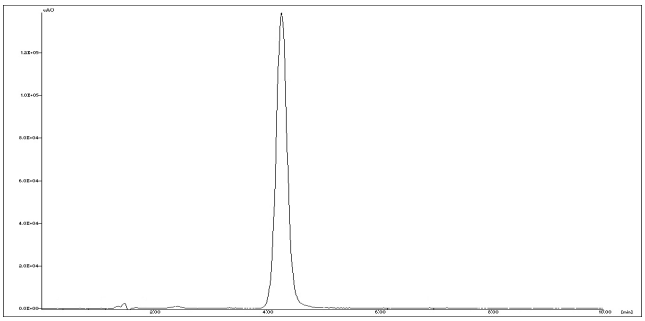

Figure 9: Vandetanib (10 µg/mL) after photodegradation

Oxidative Degradation

A 1-mL of the vandetanib working standard mixture of (1000 µg/mL) was mixed with 1-mL of 6% H_2O_2 solution then left in a dark environment for 24 hours. A 10 mL adjustment was made to the solution's volume and a milliliter of this adjusted solution was diluted even more with the mobile phase to yield a 10 µg/mL concentration. This mixture was then run under pre-determined chromatographic conditions. A percent recovery of 95.52% was achieved for vandetanib, with no degradation peak visible on the chromatograph. For chromatograms, refer to Figures 7A and 7B.

Degradation from dry heat

Dry heat degradation investigations were conducted by subjecting the drug sample to an oven at 100°C for two hours. The sample was then collected and processed following the standard solution preparation procedure, resulting in a concentration of 100 µg/mL. A mobile phase was used to further dilute the resultant solution by 1-mL to achieve a concentration of 10 µg/mL before being injected under the optimized chromatographic conditions. Vandetanib exhibited a percent recovery of 98.91% with no observable degradation product peak. For chromatograms, refer to Figure 8.

- *Photo degradation studies*

Photolytic investigations were conducted where the drug sample was subjected to UV light at a strength of up to 200 watts/hour/m^2. This was followed by exposure to a cool fluorescent light to obtain an illumination of 1.2 million Lux hours. After exposure, the sample was processed to obtain a solution concentration of 100 µg/mL. In 1-mL of the solution was further diluted with the mobile phase to achieve a final volume of 10 mL, which results in a concentration of 10 µg/mL. This solution was then run under pre-determined chromatographic conditions. Following the photodegradation study using UV and fluorescent light, there was no observable peak indicating degradation of vandetanib, and the percent recovery was determined to be 99.26% recovery. For

Table 12: Overview of stress degradation product of vandetanib

S. No.	*Stress degradation condition*	*%Recovery*	*RT of degraded product*	*Resolution*
1	Base (2 N NaOH, Kept for 24 hours)	57.42	D1- RT 2.916 and D2 -RT 3.758	D2 to D1-1.91 Drug to D2-1.74
2	Acid (2 N HCl, Kept for 24 hours)	81.49	D1- RT 2.238	Drug to D1-2.86
3	6%H_2O_2 v/v (Kept for 24 hours)	95.52	--	
4	Dry heat (100°C for 2 hours)	98.91	--	
5	Photostability [UV, 200 Watt Hours/square meter Florescence, 1.2 million Lumen hours]	99.26	--	

chromatograms, refer to Figure 9.

Summary of stress degradation product of vandetanib (Table 12).

Identification, Characterization, and Prediction of Degradation Products using LC-MS

Following the International Council of Harmonization (ICH) guidelines Q1A (R2), forced degradation studies were performed. All stressed solid samples and solutions were securely shielded using aluminum foil and stored in a deep freeze at -70°C, ensuring the preservation of the sample integrity until the analysis was undertaken. Both the standard drug and the forced degradation samples, where degradation was noted during the stability-indicating assay method's development, underwent liquid chromatography-mass spectrometry (LC-MS) under acidic and alkaline conditions.[9]

A successful partition of one degradation product in acidic conditions (Figures 10A and 10B) and two in alkaline conditions (Figures 11(A, B, C), 12, and 13) was attained using the software high-performance liquid chromatography (HPLC)

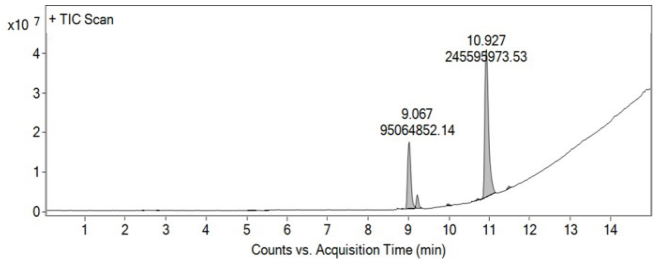

Figure 10A: Vandetanib subjected to acidic stress (One Degradant peak D1- 9.067 minutes)

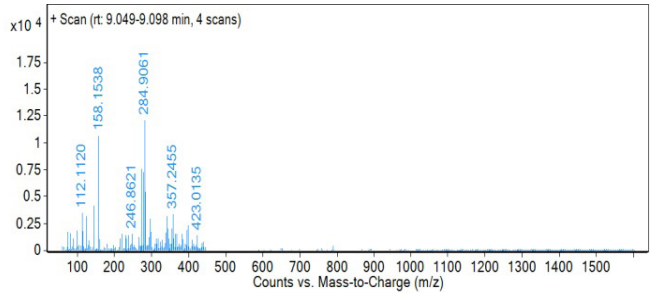

Figure 10B: The LC-MS spectrum of vandetanib's D1 acidic stress sample, which retained a time of 9.067 minutes

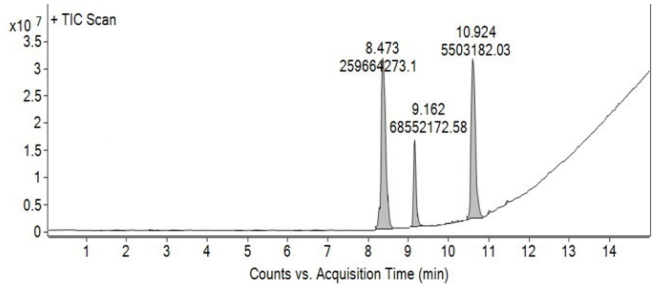

Figure 11A: Alkaline stress analysis of vandetanib exhibiting two degradation peaks - D1 (Retention Time: 9.162 minutes) and D2 (Retention time: 8.473 minutes)

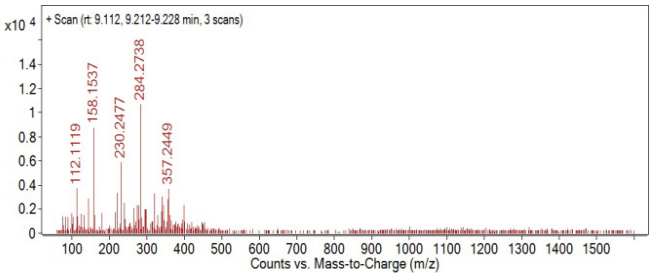

Figure 11B: The LC-MS spectrum of vandetanib's D1 alkaline stress sample was recorded, featuring a retention time of 9.162 minutes

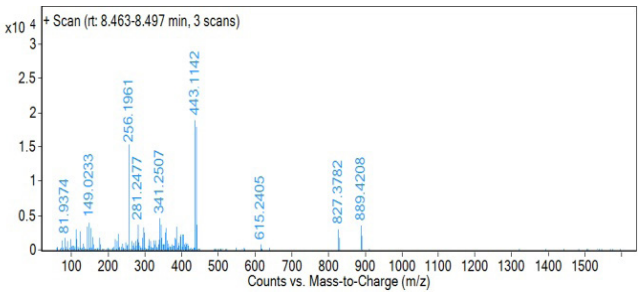

Figure 11C: The LC-MS spectrum of vandetanib's D1 alkali stress sample, which retained a time of 8.473 minutes

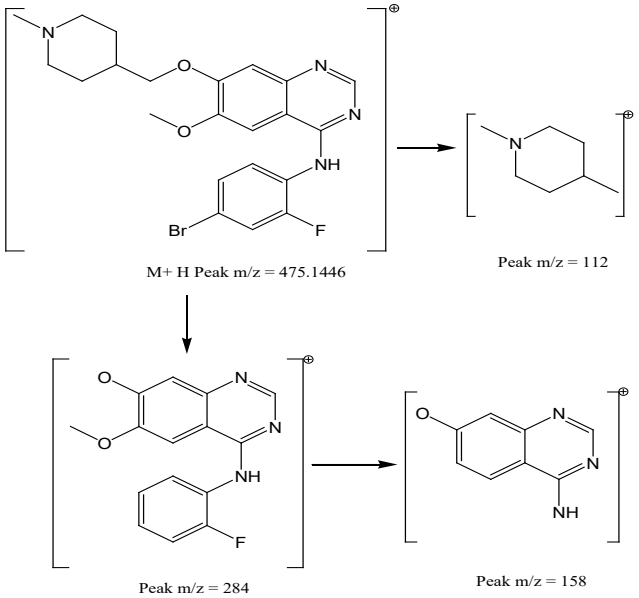

Figure 12: Probable degradation pathways for degradant D1 of vandetanib under acid/alkali stress sample

coupled with an Agilent HPH-C18 (4.6 × 100 mm, 1.7 µ) analytical column. Liquid chromatography-electrospray mass spectrometry (LC-ESI/MS) was then employed to identify and characterize these degradation products, offering accurate mass measurements up to the fourth decimal point.[10-12]

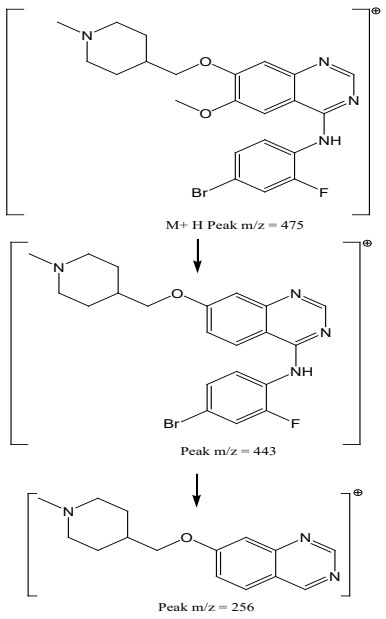

Figure 13: Probable degradation pathways for degradant D2 of vandetanib under acid/alkali stress sample

Mass spectroscopy conditions

Instrument: Agilent 6540 UHD Accurate-mass Q-TOF MS connected to Agilent 1260 infinity II HPLC
Ion source: Dual AJS ESI
Mass range (m/z): 50 to 1700
Polarity: Positive

Ion source parameters

Gas temp: 300° degrees
Gas flow: 81/minute
Nebulizer gas: 35 psig
Sheath gas temperature: 350°C degrees
Sheath gas flow: 111/minute
Capillary voltage: 3500V
Nozzle voltage: 1000 V
Column: Agilent HPH C-18, 4.6×100 mm, 1.7 micron
Column Temp: RT
Mobile phase A: 0.2% Formic acid in water
Mobile phase B: 0.2% Formic acid in acetonitrile
Rate of flow: 0.5 mL/minute

Gradient

Time (minutes): Mobile Phase B (%): 0 minutes: 20% B, 1 minutes: 30% B, 13 minutes: 100% B, 14 minutes:100% B, 15 minutes: 20% B
Post run: 20% B for 4 minutes

CONCLUSION

A highly efficient and precise HPLC method was successfully developed and authenticated to evaluate vandetanib in raw and tablet forms. The separation operation was conducted on a Grace C18 (250 × 4.6 mm, 5 µm) column. The mobile phase was a composition of Methanol: 0.1% HCOOH, in a ratio of 90:10 v/v, operating at a 1-mL/min flow rate, and detection was executed at 249 nm. The retention period (RT) for Vandetanib was established as 4.213 ± 0.067 minutes. Validation of the procedure was employed, encompassing parameters such as linearity, range, method precision (intraday and inter-day), accuracy, and robustness. The linear regression analysis exhibited an outstanding linear relationship within the concentration spectrum of 2 to 12 µg/mL, having a correlation coefficient (R^2) of 0.9985. The %assay was determined to be 100.221 ± 0.497. Vandetanib demonstrated sensitivity toward acidic, basic, and oxidative conditions. However, it exhibited photostability and thermal stability, and the sample recovery rate was found to be satisfactory. In summary, the devised technique worked as intended and proved to be straightforward, sensitive, accurate, and precise for the evaluation of vandetanib. It is appropriate for routine analysis of vsandetanib in both raw and drug dosage forms.

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AUTHOR CONTRIBUTION

An equal participation by all authors.

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