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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DASATINIB AND LENVATINIB BY USING UPLC IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

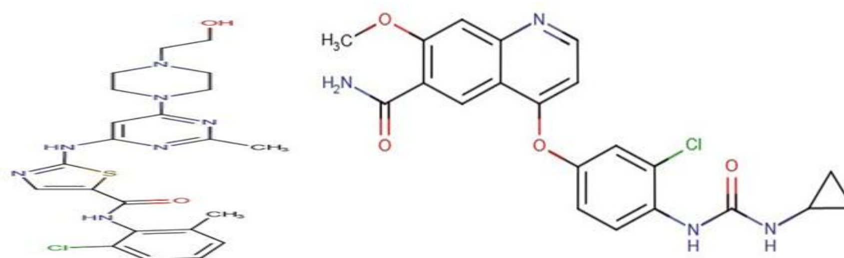
Objective: The objective of the method was to develop a new, simple, rapid, efficient, cost effective and reproducible, stability indicating Ultra performance liquid chromatography method for quantification of Dasatinib and Lenvatinib in pharmaceutical dosage form as per ICH guidelines. **Methods:** Chromatographic analysis was carried out by using UPLC on a water acquity C8 column using a mobile phase sodium phosphate buffer (10mm), pH 3.5 methanol [60:40] was selected as good peak symmetry and resolution between peaks was observed. The flow rate of 0.5ml/min was maintained and detection was carried out at 276nm. The method was validated in terms of linearity, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines. Results: The Retention time of DASATANIB and LENVATINIB were found to be 2.355 and 4.460 min respectively. The From linearity the correlation coefficient R^2 value was found to be 0.991 for DASATANIB and 0.990 for LENVATINIB. The proposed UPLC method was also valid Dasatanib for system suitability, system precision and method precision. The % RSD in the peak area of drug was found to be less than 2%. The limit of detection of DASATANIB and LENVATINIB were found to be 0.72µg/ml µg/mL and 3.19 µg/mL and limit of quantitation were 2.20µg/mL for dasatinib and 9.67 µg/ml respectively, The percentage of recovery of DASATANIB and LENVATINIB were found to be 98.74 and 99.42 respectively. The drugs were degraded in thermal, photolytic, acidic, basic and peroxide conditions. The peaks of degraded products were well resolved from the actual drug. The results obtained prove that the developed method is a

stability indicating method. Conclusion: The developed RP-HPLC method was simple, rapid, accurate, precise and stability indicating for the dasatinib and lenvatinib in pharmaceutical dosage form.

KEYWORDS: Dasatinib and Lenvatinib; UPLC; Validation; Stability indicating.

INTRODUCTION

Dasatinib is an oral dual BCR/ABL and Src family tyrosine kinase inhibitor approved for use in patients with chronic myelogenous leukemia (CML). The main targets of Dasatinib, are BCRABL, SRC, Ephrins and GFR. Whose IUPAC name is N-(2-chloro-6-methylphenyl)-2-({6-[4-(2-hydroxyethyl) piperazin-1-yl]-2-methylpyrimidin-4- yl}amino)-1,3-thiazole Carboxamide with molecular weight of 488.006. Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Based on modeling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. In vitro, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Lenvatinib is indicated for the treatment of patients with locally recurrent or metastatic, progressive, radioactive iodine (RAI)-refractory differentiated thyroid cancer. IUPAC name**:**4-{3-chloro-4-[(cyclopropylcarbamoyl)amino]phenoxy}-7-methoxyquinoline-6-carboxamide with molecular formula 426.86. Lenvatinib is a receptor tyrosine kinase (RTK) inhibitor that inhibits the kinase activities of vascular endothelial growth factor (VEGF) receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4). Lenvatinib also inhibits other RTKs that have been implicated in pathogenic angiogenesis, tumor growth, and cancer progression in addition to their normal cellular functions, including fibroblast growth factor (FGF) receptors FGFR1, 2, 3, and 4; the platelet derived growth factor receptor alpha (PDGFRα), KIT, and RET.), KIT, and RET.



(A) Dasatinib (B) Lenvatinib

Fig. 1: (A) and (B).

MATERIALS AND METHOD

Instrument Used

UV-Visible Spectrophotometer-Thermo Electron co-orporation, UPLC-Agilent Infinity 1290, Ultra Sonication -Citizen, Digital Ultrasonic Cleaner, pH meter-Thermo, Electronicbalance-Mettler Toledo ,Syringe-Hamilton ,UPLC Column-Waters Acquity C8 125A°(100x2.2mm ID) 1.7µm.

Drugs Used

Dasatinib and Lenvatinib bulk drugs-Gift samples obtained from Madras pharmaceuticals, Chennai, and Dasatinib and Lenvatinib –pharmacy.

Reagent and Solutions

Methanol, Acetonitrile, Water (UPLC grade), Sodium hydroxide.

Determination of Working Wavelength

Preparation of standard stock solution of Dasatinib

10 mg of DASATINIB was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Lenvatinib

10 mg of Lenvatinib was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank.

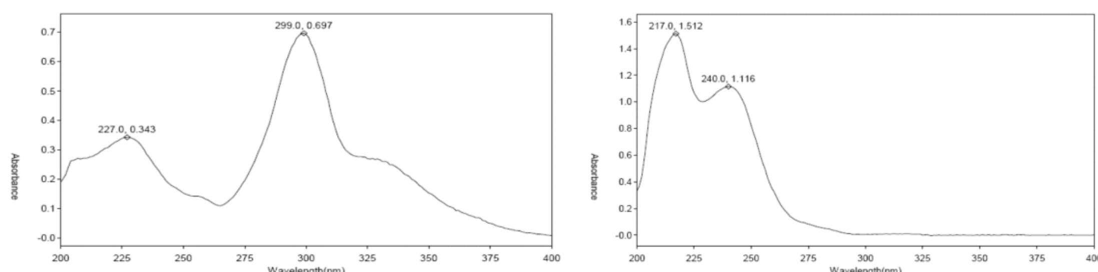


Fig. 1: UV-VIS Spectrum of Dasatinib 299 nm Fig: UV-VIS Spectrum of Lenvatinib240 nm

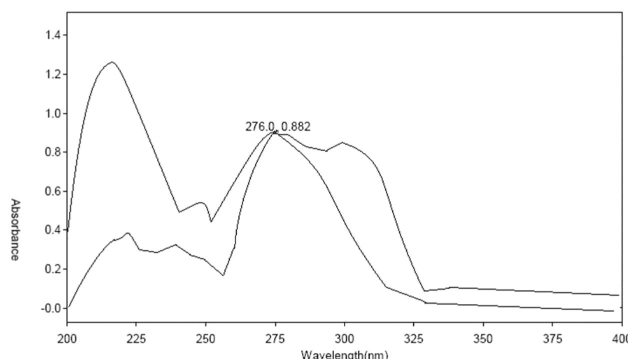


Fig. 3: Isobestic point 276 nm.

RESULT AND DISCUSSION

Trails were performed for the method development and thebest peak with least fronting factor to be with RT= 2.355 for dasatinib and 4.460 min for Lenvatinib.

Optimized Chromatographic Conditions (Table-1).

Mobile phase	Sodium Phosphate buffer(10mm) ,pH 3.5:Methanol [60:40]
Column	Waters Acquity C8 125A°(100x2.2mm ID) 1.7µm
Flow rate	0.5 mL/min
Column temperature	30°C
Wavelength	276 nm
Injection volume	10µL
Run time	5.0 min

Validation

1. System Suitability

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 50µg/mL of DESATINIB and 70µg/mL of LENVATINIB were injected six times and the chromatograms were recorded for the same.

Table 1: Results for system suitability of Desatinib.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.355	1167.33	31425	1.07
2	2.356	1185.69	31155	1.08
3	2.357	1186.47	31384	1.07
4	2.356	1190.47	31308	1.06
5	2.354	1189.12	31372	1.07
6	2.356	191.96	32851	1.06
Mean	2.357	1194.21	-	-
SD	0.03	11425	-	-
%RSD	0.1	1.4	-	-

Table 2: **Results for system suitability of Lenvatinib.**

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.460	1313.00	23819	1.08	7.4
2	4.462	1314.51	23895	1.07	7.5
3	4.461	1312.58	23874	1.06	7.4
4	4.463	1317.21	23871	1.05	7.3
5	4.465	1315.21	23796	1.04	7.2
6	4.460	1314.25	23714	1.07	7.5
Mean	4.467	1317.25	-	-	-
SD	0.04	8465	-	-	-
%RSD	0.6	0.7	-	-	-

RESULT

The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

SPECIFICITY: Blank solution was injected and the chromatogram was recorded for the same as given in Fig. Placebo solution was prepared and it was injected and the chromatogram was recorded for the same as given in Fig.

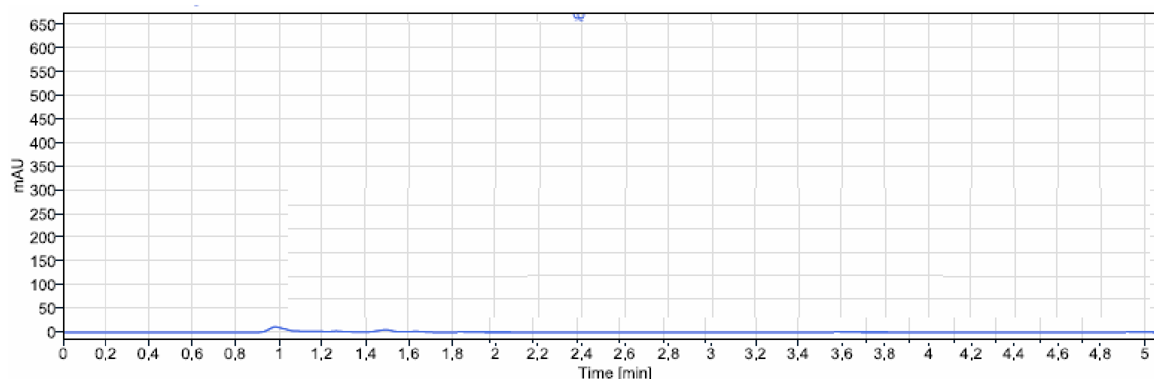


Fig. 1: **Chromatogram of Desatinib and Lenvatinib Blank.**

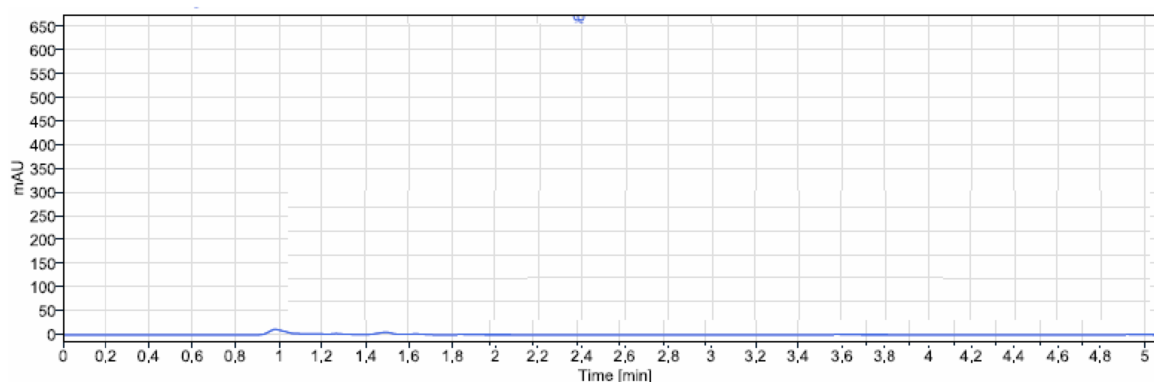


Fig. 2: **Chromatogram of Placebo.**

RESULT

It was observed that diluent or placebo peaks was not interfering with the DESATINIB and Lenvatinibpeaks.

Linearity and Range

Preparation of standard stock solution

Standard stock solutions of DESATINIB (500µg/mL) and LENVATINIB (700µg/mL) were prepared by dissolving 50 mg of DESATINIB and 50 mg of LENVATINIB in 100 mL of mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min further dilutions were given in the Table1.

Preparations	Volume from standard stock transferred in mL	Volume made up in mL (with mobile phase)	Conc. obtained (µg/mL)	
			DASATINIB	LENVATINIB
Preparation 1	0.2	10	20	20
Preparation2	0.3	10	30	30
Preparation 3	0.4	10	40	40
Preparation 4	0.5	10	50	50
Preparation 5	0.6	10	60	60

Table 2: Linearty Data of Dasatinib.

S. No	Concentration (µg/mL)	Area
1	20	2449.75
2	30	3500.89
3	40	3556.99
4	50	4918.92
5	60	7842.69

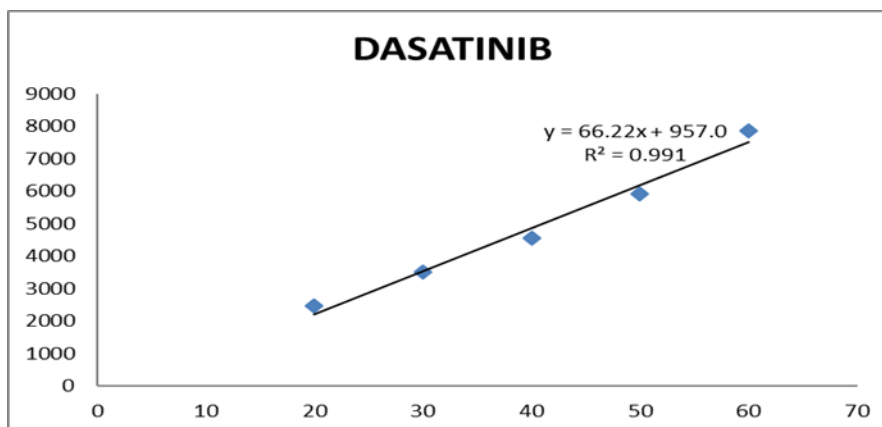


Fig. 3: Graph for Linearity data of Dasatinib.

Table-3:

S. No	Concentration (µg/mL)	Area
1	20	1452.33
2	40	3890.02
3	60	5543.79
4	80	7393.69
5	100	9074.45

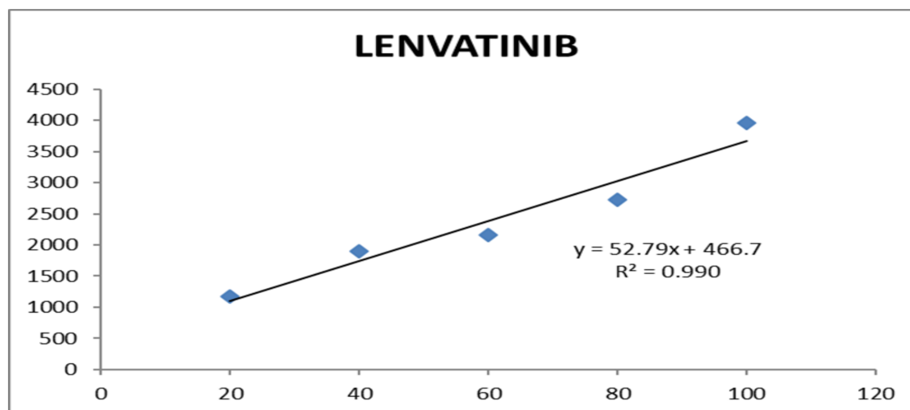


Fig. 4: linearty graph of lenvatinib

Table 4: Observation for linearity.

S.No	Parameter	DASATINIB	LENVATINIB
1	Correlation coefficient	0.991	0.990
2	Slope	66.22	52.79

RESULT

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of DASATANIB and LENVATINIB is 0.991 and 0.990 respectively.

ACCURACY

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table.

***Table* 1: Results for Recovery of DESATINIB.**

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	% Mean Recovery
50%	25	25.1	100.1	100.3
100%	50	49.90	99.8	
150%	75	75.2	100.4	

* Mean of three observations

***Table 2*: Results for Recovery of Lenvatinib.**

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	% Mean Recovery
50%	35	35.1	100.1	100.2
100%	70	70.4	100.3	
150%	105	105.1	100.2	

* Mean of three observations

Result The % mean recovery of DESATINIB and LENVATINIB was founded between 98.0 to 102.0.

METHOD PRECISION

Method precision was determined by injecting six different solutions of sample solutions DESATINIB (50 µg/mL) and LENVATINIB (70µg/mL) for six timesare prepared separately.

***Table*: Method precision results for Desatinib and Lenvatinib.**

Injection	DESATINIB		LENVATINIB	
	Area	%Assay	Area	%Assay
1	3189.91	100.2	1313.90	99.4
2	3188.60	100.0	1313.88	99.2
3	3184.27	99.7	1314.68	99.5
4	3184.54	99.2	1314.40	99.3
5	3186.15	99.4	1314.23	99.4
6	3189.21	98.7	1314.55	99.1
Average	-	99.4	-	99.2
SD	-	0.8	-	0.6
%RSD	-	0.8	-	0.6

Result The %RSD of 6 determinations of DESATINIB and LENVATINIB for System precision found to be within the acceptance criteria of less than 2.0%.

Limit of Detection

$$LOD = \frac{3.3\sigma}{S}$$

= (3.3)*(19.03)/87.38

= 0.72µg/ml

= (3.3)* (26.65)/27.56

=3.19µg/ml

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

OBSERVATION

The LOD for this method was found to be 0.72µg/ml for DESATINIB and 3.19µg/ml for Lenvatinib

Limit of Quantification

$$LOQ = \frac{10\sigma}{S}$$

= (10)*(19.03)/87.38

= 2.20µg/ml

= (10)* (26.65)/27.56

=9.67µg/ml

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOQ for this method was found to be 2.2µg/ml for DESATINIB and 9.67µg/ml for Lenvatinib.

Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table 1

Chromatographic changes		Theoretical Plates		Tailing factor		Resolution
		DES	LEV	DES	LEV	Between DES&LEV
Flow rate (mL/min)	0.4	11241	11365	1.01	1.11	7.5
	0.6	11458	11362	1.06	1.06	7.1
Temperature(°C)	20	11425	11947	1.09	1.04	7.9
	30	11574	10958	1.07	1.03	7.5

Result-The tailing factor was found to be within the limits on small variation of flow rate and Temperature.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Table Result of ruggedness.

DESATINIB	%Assay	LENVATINIB	%Assay
Analyst 01	99.4	Analyst 01	100.1
Anaylst 02	99.3	Anaylst 02	100.2
% RSD	0.2	% RSD	0.1

Observation From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Forced Degradation studies

The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, is carried out to demonstrate as specificity to developed a stability-indicating analytical method, using HPLC or UPLC i.e., a single analytic method that is capable of separating the degradant peaks from the drug substance/drug product peak. As per International Conference on Harmonization (ICH) guidelines (Q1A), stability studies need to be performed to propose the shelf life of new drug substances and/or drug products. Shelf life studies are part of various regulatory submissions to the FDA.

Table Result of stability studies.

Injection		DESATINIB	LENVATINIB
	Condition	%Assay Area	%Assay
1	Thermal	98.1	99.3
2	Photolytic	98.9	100.3
3	Acid Hydrolysis	97.5	97.1
4	Base Hydrolysis	99.9	99.1
5	Peroxide Hydrolysis	96.5	98.7

CONCLUSION

1. The optimum wavelength for the determination of DASATANIB and LENVATINIB was selected at 276 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but Sodium Phosphate buffer(10mM)_pH 3.5: : Methanol [60:40] was selected as good peak symmetry and resolution between the peaks was observed.

2. The Retention time of DASATANIB and LENVATINIB were found to be 2.355 and 4.460 min respectively. The retention times for both the drugs were considerably less compared to the retention time obtained for the drugs in the other mobile phase.
3. The different analytical performance parameters such as linearity, precision, accuracy, and specificity, LOD, LOQ were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 60-140 µg/mL For DASATANIB and 12-28 µg/mL for LENVATINIB.
4. From linearity the correlation coefficient R^2 value was found to be 0.999 for DASATANIB and 0.999 for LENVATINIB. The proposed HPLC method was also valid Dasatanibd for system suitability, system precision and method precision. The % RSD in the peak area of drug was found to be less than 2%. The number of theoretical pl Dasatanib was found to be more than 2000, which Dasatanib efficient performance of the column.
5. limit of detection of DASATANIB and LENVATINIB were found to be 0.72µg/ml µg/mL and 3.19 µg/mL and limit of quantitation were 2.20µg/mL for dasatinib and 9.67 µg/ml respectively, The percentage of recovery of DASATANIB and LENVATINIB were found to be 98.74 and 99.42 respectively shows that the proposed method is highly accurate.
6. The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies.

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