International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

RESEARCH ARTICLE

Novel RP-HPLC Method Development and Validation of Dasatinib and Lenvatinib in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A new method was established for simultaneous estimation of Dasatinib and Lenvatinib by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Dasatinib and Lenvatinib by using ThermosilC18 column (4.0×125mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Dasatinib and Lenvatinib was found to be 101.27% and 99.97% respectively. The system suitability parameters for Dasatinib and Lenvatinib such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Dasatinib and Lenvatinib was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC.

Keywords: Thermosil C18 column, Dasatinib and Lenvatinib, RP-HPLC

ARTICLE INFO

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MS-ID: IJCTPR3738

PAPER QR-CODE

Article History: Received 31 January 2019, **Accepted** 25 February 2019, **Available Online** 15 March 2019

Citation: K. Nageswara Rao, *et al.* Novel RP-HPLC Method Devlelopment and Validation of Dasatinib and Lenvatinib in Bulk and Pharamceutical Dosage Forms.*Int. J. Curnt. Tren. Pharm, Res., Res*., 2019, 7(2): 50-56.

CONTENTS

1. Introduction

Dasatinib is an oral medication used for treating chronic myeloid leukemia and acute lymphoblastic leukemia. It is classified as a kinase inhibitor. Kinase inhibitors prevent the growth of tumors by reducing the action of proteins that control cell division, growth and survival. These proteins are usually present in larger quantities or are more active in cancer cells. By reducing the activity of these proteins, growth and survival of cancer cells are reduced. The chemical name for dasatinib is N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5- thiazolecarboxamide monohydrate the molecular formula is C22H26ClN7O2S.H2O, which corresponds to a formula weight of 506.02 (monohydrate). The anhydrous free base has a molecular weight of 488.01. Dasatinib is a white to off-white powder and has a melting point of 280°- 286°C. The drug substance is insoluble in water and slightly soluble in ethanol and methanol. Dasatinib is an inhibitor of multiple tyrosine kinases. Researchers found proof that few analytical methods such as HPLC, LC-MS and UPLC methods have been reported for the estimation of dasatinib.

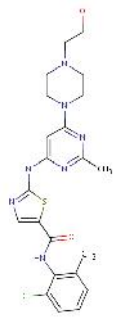

Figure 1: Structure of Dasatinib

Lenvatinib is a multiple receptor tyrosine kinase (RTK) inhibitor indicated for the treatment of thyroid cancer. Lenvatinib restrains kinase activities of vascularndothelial growth factor receptors. It also simultaneously restrains other receptors involved in the tumour angiogenesis and proliferation of thyroid cancer including fibroblast growth factor and the platelet derived growth factor receptor alpha. Lenvatinib is chemically known as 4-[3-chloro–4-(cyclopropyl carbamoyl amino)phenoxy]-7-methoxyquinoline–6–carboxamide.Researchers reveals that very few analytical methods have been reported for the determination of lenvatinib which includes high performance liquid chromatography, Liquid chromatography-mass spectroscopy and pharmacokinetics studies.

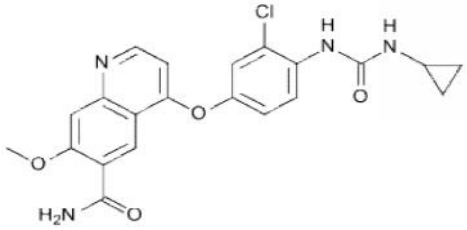

Figure 2: Structure of Lenvatinib

Literature review reveals that no analytical methods have been reported for the determination of simultaneous estimation of dasatinib and lenvatinib drugs which includes RP-HPLC in pharmaceutical dosage form. The present study was aimed to develop a simple and accurate RP-HPLC method for the estimation of dasatinib and lenvatinib drug according to ICH guidelines.

2. Materials and Methods

Materials

Dasatinib, Lenvatinib, Kh_2PO4,Water And Methanol For HPLC, Acetonitrile for HPLC, Ortho Phosphoric Acid.

Instruments

HPLC, WATERS, software: Empower, 2695 separation module, PDAdetector. UV/VIS spectrophotometer LAB INDIA UV 3000^{+},pH meter Adwa – AD 1020,Weighing machine Afcoset ER-200A.

Method development for the simultaneous estimation of Dasatinib and Lenvatinibby using RP-HPLC.

1. Selection of mobile phase
2. Selection of detection wavelength
3. Selection of column
4. Selection of solvent delivery system
5. Selection of flow rate
6. Selection of column temperature
7. Selection of diluent
8. Selection of test concentration and injection volume

Selection of mobile phase

- Sodium acetate buffer : Methanol (30 : 70% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
- Above 8: dissolution of silica.
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.
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Selection of wavelength: 10 mg of Dasatinib and Lenvatinib was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Dasatinib and Lenvatinib. The isobestic point was taken as detection wavelength.

Preparation of mobile phase:

Mix a mixture of above buffer 30 ml (30%) and 70 ml of Methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Sample solution preparation:

1mg of Lenvatinib and 10 mg Dasatinib tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml

of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

1mg Lenvatinib and 10 mg Dasatinib in working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity studies should cover the range of 0-150% of the expected level of the analyte. The data is then processed using the method of least squares regression. The resulting plot, slope, intercept and correlation coefficient provide the desired information on linearity. ICH recommends that, for the establishment of linearity, a minimum of five concentrations should normally be used

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

a. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

b. Intermediate precision: Intermediate precision expresses within laboratories variations: different day's different analysts, different equipment, etc.

c. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range of the procedure (i.e., three replicates of three concentrations) or using a minimum of six determinations at 100% of the test concentration.

Detection Limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the Quantitationn limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range of the analytical procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy and linearity when applied to the samples containing analytes at the extremes of the range as well as within the range.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. A good practice is to vary important parameters in the method systematically and measure their effect on separation. The variable method parameters may involve temperature (±50C), buffer pH (±0.5), ionic strength of buffers, level of additives to MP, flow rate (±0.2ml/min), wavelength (±2nm).

Ruggedness

The precision obtained when the assay is performed by multiple analysis, using multiple instruments, on multiple days, in one laboratory, different sources of reagents and multiple lots of columns should also be included in this study.

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. The accuracy and the precision of HPLC data collected, which begins with a well -behaved chromatographic system. The system suitability parameter and tests are the parameters that help in achieving this purpose.

3. Results and Discussion

Table 1: Optimized chromatographic conditions

Column	**Thermosil C18 (4.0×125mm)5.0µm**
Mobile phase ratio	Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength	252 nm
Flow rate	0.7 ml/min
Injection volume	10µl
Column temperature	Ambient
Auto sampler temperature	Ambient

Run time	8 min
Retention time	2.449 & 3.191 mins

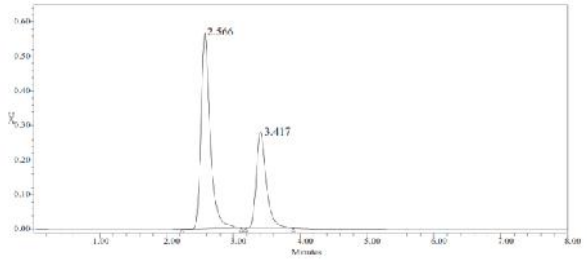

Figure 3: Optimized Chromatogram

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Linearity:

The linearity study was performed for concentration range of 5.µg-25µg and 50µg-250µg of Dasatinib and Lenvatinib and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999).

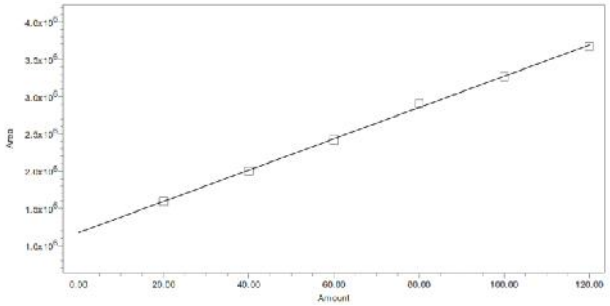

Figure 4: Showing calibration graph for Dasatinib

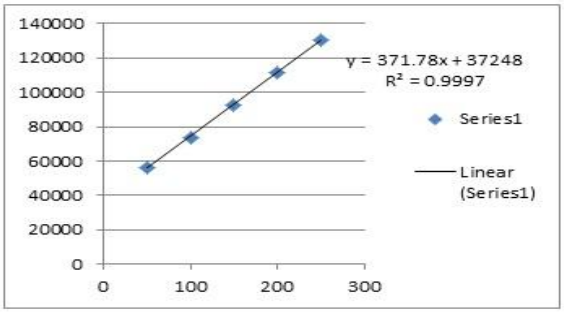

Figure 5: Showing calibration graph for Lenvatinib

Accuracy: The accuracy study was performed for % recovery of Dasatinib and Lenvatinib. The % recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%).

Precision:

The Method precision study was performed for the %RSD of Dasatinib and Lenvatinib was found to be 0.82 and 0.86 (NMT 2).

Intermediate precision: The intermediate precision was performed for %RSD of Dasatinib and Lenvatinib was found to be 0.19 and 0.44 respectively (NMT 2).

Limit of Detection:

The LOD was performed for Dasatinib and Lenvatinib was found to be 3.17and 0.0172 respectively.

Limit of Quantification:

The LOQ was performed for Dasatinib and Lenvatinib was found to be 5.80 and 0.212 respectively.

Robustness:

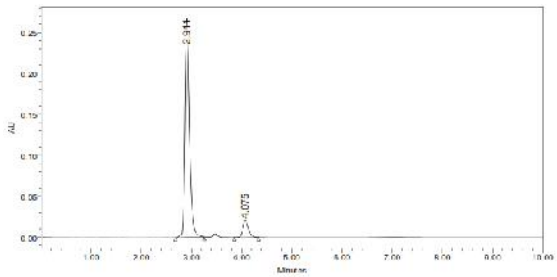

Figure 6: Chromatogram showing less flow rate 0.8ml/min

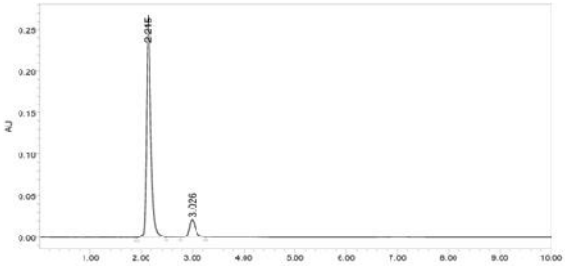

Figure 7: Chromatogram showing more Flow rate 1.2 ml/min

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

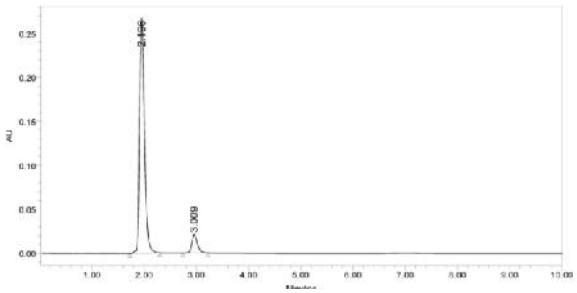

Figure 8: Chromatogram showing more Organic phase ratio

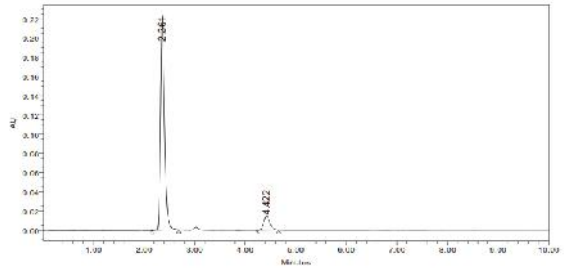

Figure 9: Chromatogram showing less Organic phase ratio

On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

4. Conclusion

A new method was established for simultaneous estimation of Dasatinib and Lenvatinib by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Dasatinib and Lenvatinib by using ThermosilC18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate bufferpH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Dasatinib and Lenvatinib was found to be 101.27% and 99.97% respectively. The system suitability parameters for Dasatinib and Lenvatinib such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Dasatinib and Lenvatinib was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Dasatinib and Lenvatinib in API and Pharmaceutical dosage form.

Table 2: Results of system suitability parameters for Dasatinib and Lenvatinib

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Dasatinib**	2.5	124505	213642		1.2	4673.4
2	**Lenvatinib**	3.9	1308495	154566	6 0	1.3	6090.3

Table 3: Linearity Results for Dasatinib

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
2	II	10 ppm	656277
3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient		0.999	

Table 4: Linearity Results for Lenvatinib

S.No	Linearity Level	Concentration	Area
1	I	50ppm	56472
2	II	100 ppm	73841
3	III	150ppm	92655
4	IV	200ppm	111541
5	V	250ppm	130567
Correlation Coefficient		0.999	

Table 5: Showing accuracy results for Dasatinib

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	
100%	5277055	10	9.98	99.18%	99.56%
150%	7514836	15	15.02	99.60%	

Table 6: Showing accuracy results for Lenvatinib

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	
100%	2777487	1.0	1.05	99.38%	99.47%
150%	4151234	1.5	1.495	99.52%	

Table 7: Showing %RSD results for Dasatinib and Lenvatinib

Peak Name: Dasatinib

	Peak Name	RT	Area	Height (µV) 1
	Dasatinib	3.616	2742453	238643.4
2	**Dasatinib**	3.634	2762750	271543.5
3	**Dasatinib**	3.460	2797670	281711.6
4	**Dasatinib**	3.446	2793578	274499.8
5	**Dasatinib**	3.437	2778483	276713.0
Mean			2774987	
Std. Dev.			22806.9	
% RSD			0.82	

Peak Name: Lenvatinib

	Peak Name	RT	Area	Height (µV) 1
	Lenvatinib	2.755	5223539	541338.3
2	**Lenvatinib**	2.687	5208511	485548.5
3	**Lenvatinib**	2.632	5323509	574440.4
4	**Lenvatinib**	2.612	5259147	557413.5
5	**Lenvatinib**	2.616	5273463	565020.1
Mean			5257650	
Std. Dev.			45206.4	
% RSD			0.86	

Table 8: Showing intermediate precision results for Dasatinib and Lenvatinib

Peak Name: Dasatinib

	Peak Name	RT	Area	Height (µV) 1
	Dasatinib	3.617	2624315	231325.6
2	**Dasatinib**	3.635	2623598	231315.4
3	**Dasatinib**	3.461	2623541	231250.1
4	**Dasatinib**	3.447	2624987	231342.6
5	**Dasatinib**	3.438	2635698	231765.2
Mean			2626428	
Std. Dev.			5215.78	
% RSD			0.19	

Peak Name: Lenvatinib

	Peak Name	RT	Area	Height (µV)
1	**Lenvatinib**	2.756	5698542	539568.1
2	**Lenvatinib**	2.688	5682534	536985.4
3	**Lenvatinib**	2.633	5695846	539584.1
4	**Lenvatinib**	2.613	5689452	534569.8
5	**Lenvatinib**	2.617	5636591	534985.5
Mean			5600593	
Std. Dev.			203577.3	
% RSD			0.44	

Table 9: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Dasatinib	373625.50	581075863	3.17
Lenvatinib	5772.40	476579210	0.0172

Table 10: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Dasatinib	372727.80	574265980	5.80
Lenvatinib	5761.30	478828490	0.212

Table 11: Showing system suitability results for Dasatinib

S.No	**Flow rate (ml/min)**	**System suitability**	
		USP Plate count	USP Tailing
1	0.8	5339	1.4
2	1	4668	1.3
3	1.2	5216	1.4

Table 12: Showingsystem suitability results for Lenvatinib

S.No	**Flow rate (ml/min)**	**System suitability**	
		USP Plate count	USP Tailing
1	0.8	7036	1.3
2	1	6089	1.2
3	1.2	6998	1.3

Table 13: Showing system suitability results for Dasatinib

S.No	**Change in organic composition in the mobile phase**	**System suitability**	
		USP Plate count	USP Tailing
1	5% less	6232	1.4
2	Actual	4668	1.3
3	5% more	6387	1.4

Table 14: Showing system suitability results for Lenvatinib

S.No	Change in organic composition in the mobile phase	System suitability	
		USP Plate count	USP Tailing
1	5% less	5437	1.3
2	Actual	6089	1.2
3	5% more	4817	1.2

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