International Journal of Pharmacy and Natural Medicines

Journal Home Page: www.pharmaresearchlibrary.com/ijpnm

R E S E A R C H A R T I C L E

RP-HPLC Method Development and Validation for Simultaneous Estimation of Dasatinib and Lenvatinib in Its active Pharmaceutical Ingredient and Pharmaceutical Dosage Forms

B. Yamuna Rani[1], Dr. D. Naresh[2], Dr. Gampa Vijay Kumar[3*]

[1]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[2]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[3]*Professor and Head, Dept. of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*

A B S T R A C T

A new method was established for simultaneous estimation of a Dasatinib and Lenvatinib by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Dasatinib and Lenvatinib by using Hypersil ODS C18 5µm (4.6*250mm) column, flow rate was 0.8 ml/min, mobile phase ratio was (30:75 v/v) acetonitrile: phosphate buffer (KH_2PO_4 and K_2HPO_4)pH 3.0 (pH was adjusted with orthophosphoric acid), detection wave length was 255 nm. The instrument used was Shimadzu, UV detector, LC solutions. The retention times were found to be 2.669 mins & 2.915mins. The % purity of Dasatinib and Lenvatinib was found to be 99.95% and 100.24%respectively. The system suitability parameters for Dasatinib and Lenvatinib such as theoretical plates and tailing factor were found to be 4668.7, 1.2 and 6090.3 and 1.3. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Dasatinib and Lenvatinib was found in concentration range of 100-500 µg/mland 1-5µg/mland correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.84%and 100.51%, %RSD for precision was 0.2 and 0.6, % RSD for intermediate precision was 0.2 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.9 and 2.3, and LOQ value was 10.03 and 10.1 respectively.

Key words: Dasatinib, Lenvatinib, RP-HPLC, Methanol, validation.

A R T I C L E I N F O

***Corresponding Author**
Dr. Gampa Vijay Kumar
KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.
MS-ID: IJPNM4074

PAPER QR-CODE

A R T I C L E H I S T O R Y: Received 05 July 2019, **Accepted** 07 Oct 2019, **Available Online** 15 December 2019

Citation: Dr. Gampa Vijay Kumar, *et al.* RP-HPLC Method Development and Validation For Simultaneous Estimation of Dasatinib and Lenvatinib In Its active Pharmaceutical Ingredient and Pharmaceutical Dosage Forms. *Int. J. Pharm. Natural Med.*, 2019, 7(2): 73-77.

C O N T E N T S

1. Introduction

Dasatinib, sold under the brand name Sprycel, is a targeted therapy used to treat certain cases of chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). Specifically it is used to treat cases that are Philadelphia chromosome-positive (Ph+).It is taken by mouth.

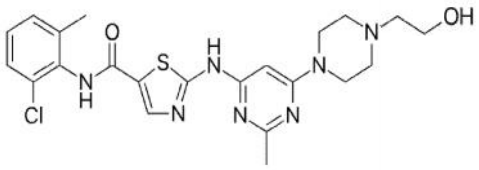

Fig 1: Structure of Dasatinib

Lenvatinib (trade name Lenvima) is an anti-cancer drug for the treatment of certain kinds of thyroid cancer, and potentially for other cancers as well. It was developed by Eisai Co. and acts as a multiple kinase inhibitor against the VEGFR1, VEGFR2 and VEGFR3 kinases.

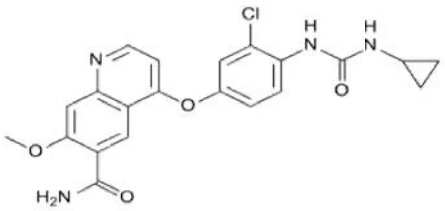

Fig 2: Structure of Lenvatinib

2. Materials and Methods

Instrumentation:

SystemAllianceWaters 2690 separation module, Pump Analytical HPLC isocratic pump,DetectorPhoto diode array detector,SoftwareEmpower 2 software,ColumnAgilent (250×4.6mm, 5µ) C-18 RP-column,Sonicator, Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometerLABINDIA, UV 3000^{+}, pH metersAD, 102U, weighing machine.

Chemicals:

Dasatinib and Lenvatinib, KH_2PO_4,Water and Methanol for HPLC,Acetonitrile for HPLC,Ortho phosphoric Acid.

Optimized chromatographic conditions

Mobile phase : AcetonitrilepH 3.0: Buffer(30:70%v/v)
Column :Hypersil ODS C18 5µm (4.6*250mm)
Flow rate : 0.8 ml/min
Wavelength : 255 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume: 10 µl

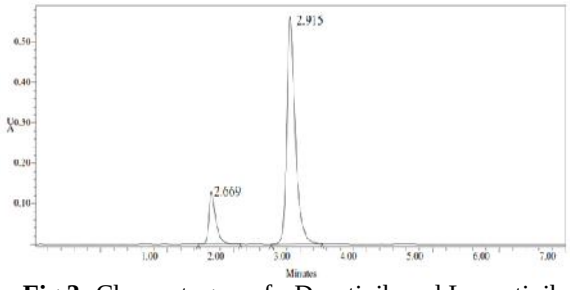

Fig 3: Chromatogram forDasatinib and Lenvatinib

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.

Standard solution preparation:

Accurately weigh and transfer 10 mg of Dasatinib and Lenvatinib10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample solution preparation:

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Dasatinib and Lenvatinib (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 3 ml of Dasatinib e and Lenvatinib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion

Table 1: Results of system suitability parameters for Dasatinib and Lenvatinib

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

Table 2: Results of method precession for Dasatinib

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Results of method precession for Lenvatinib

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 4: Results of Intermediate precision for Dasatinib

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5: Results of Intermediate precision for Lenvatinib

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Table 6: Accuracy (recovery) data for Dasatinib

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 7: Accuracy (recovery) data for Lenvatinib

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	

150%	177940	14.2	14.45	99.68%	

Table 8: Area of different concentration of Dasatinib

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	368934
2	II	200ppm	726781
3	III	300ppm	1053873
4	IV	400ppm	1463458
5	V	500ppm	1767084
Correlation Coefficient			0.999

Table 9: Area of different concentration of Lenvatinib

S.No.	Linearity Level	Concentration	Area
1	I	1ppm	36510
2	II	2ppm	74701
3	III	3ppm	106802
4	IV	4ppm	142731
5	V	5ppm	179732
Correlation Coefficient			0.999

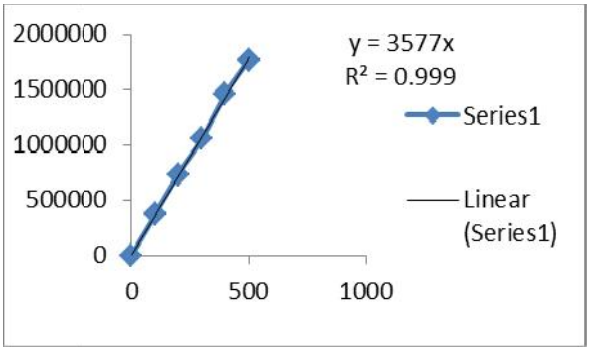

Fig 4: Calibration curve of Dasatinib

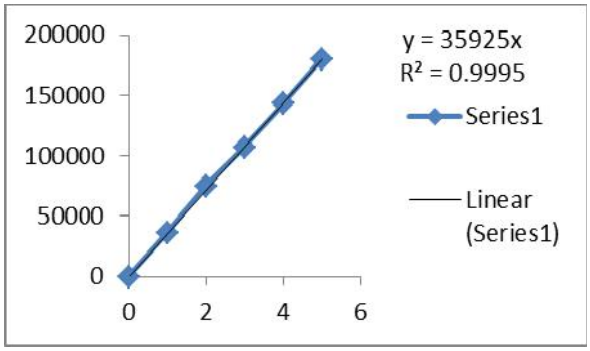

Fig 5: Calibration curve of Lenvatinib

Table 10: Analytical performance parameters of Dasatinib and Lenvatinib

Parameters	Dasatinib	Lenvatinib
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Dasatinib	52	152	2.9
Lenvatinib	52	156	3

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Dasatinib	52	522	10.03
Lenvatinib	52	524	10.1

Table 13: Flow Rate (ml/min) data for Dasatinib

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 14: Flow rate (ml/min) data for Lenvatinib

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	7063.3	1.3
2	0.8	6090.3	1.2
3	1.0	6998.0	1.3

Table 15: Change in Organic Composition in the Mobile Phase for Dasatinib

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 16: Change in Organic Composition in the Mobile Phase for Lenvatinib

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

RP-HPLC method for the estimation of Dasatinib and Lenvatinib in its active ingredient and pharmaceutical dosage form was established and validated as per the ICH guidelines. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Dasatinib and Lenvatinib in its active ingredient and pharmaceutical dosage forms.

5. References

[1] Sreedevi et al, Development And Validation Of Novel Hplc Method For The Estimation Of Dasatinib In Bulk And Pharmaceutical Dosage Forms, International Journal Of Research In Pharmacy And Chemistry Vol 11331-1345

[2] Arunkumarkalekar et al, Development and validation of Rp-hplc method for Estimation of Dasatinib in bulk and its pharmaceutical Formulation. American Journal of Pharmatech Research Vol 62231-2243

[3] Thulase NADH Reddy.Doddaet al,Method development and validation of Rp-hplc method for Estimation of Dasatinib in bulk and its pharmaceutical Formulation. Indo American Journal of Pharmaceutical Research. 2013; 3(12): 1331-1345 Vol.2

[4] Regalla Narasimha Reddy et al, Method Development And Validation Of Stability Indicating Rp-Hplc Method For The Estimation Of Dasatinib In Tablet Dosage Form, Indo American Journal of Pharmatech Research 2017 Vol.4221-1147

[5] K. K. Kumar, K. E. V. Nagoji, and R. V. Nadh et al, A Validated RP-HPLC Method for the Estimation of Lapatinib in Tablet Dosage form using Gemcitabine Hydrochloride as an Internal Standard. Indian J Pharm Sci. 2012 Nov-Dec; 74(6): 580–583. Vol 5