A new analytical novel RP-HPLC method development and validation for the quantitative determination of Dasatinib in pure form and marketed pharmaceutical dosage form

Sumera Iram [1*], Yerragolla Nuthana [2], Marella Shireesha [3], Kondoju Sai Teja [4], Somoju Sairam Chary [5]

[1] Assistant Professor, Department of Pharmacy Practice, Sree Dattha Institute of Pharmacy, Sheriguda (V), Ibrahimpatnam (M), Ranga Reddy, Telangana, India

[2-5] Department of Pharmaceutical Analysis, Sree Dattha Institute of Pharmacy, Sagar Road, Sheriguda, Ibrahimpatnam, Telangana, India

* Corresponding Author: **Sumera Iram**

Article Info

ISSN (online): 2582-8940
Volume: 05
Issue: 02
April-June 2024
Received: 08-03-2024;
Accepted: 10-04-2024
Page No: 31-37

Abstract

The present work includes a simple, economic, rapid, accurate and precise isocratic RP-HPLC method development for estimation of Dasatinib in bulk form and its marketed formulation. Estimation was done at 286nm which was found to be λmax of Dasatinib. The simple, selective, isocratic RP-HPLC method for Dasatinib was developed on Phenomenex Luna (C_{18}) RP Column; 250 mm x 4.6 mm, 5µm with a mobile phase of Phosphate Buffer (pH-4.6) and Methanol were taken in the ratio of 65:35% v/v at a flow rate of 1.0 ml/min and detection wavelength 286nm. The developed method was validated successfully according to ICH Q2 (R1) guidelines. The chromatographic methods showed a good linear response with r2 values of 0.9995. The percentage relative standard deviation for method was found to be less than two, indicating that the methods were precise. The mean percentage recovery was for RP-HPLC method was 100.437%. From the results it could be concluded that both the developed method was specific, selective and robust. The method could be successfully applied for analysis of Bulk form and Marketed formulation of Dasatinib.

Keywords: Dasatinib, RP-HPLC, Method Development, Validation, ICH Guidelines

Introduction

Dasatinib Anhydrous is an orally bioavailable synthetic small molecule-inhibitor of SRC-family protein-tyrosine kinases. Dasatinib[1] binds to and inhibits the growth-promoting activities of these kinases. Apparently because of its less stringent binding affinity for the BCR-ABL kinase, Dasatinib has been shown to overcome the resistance to imatinib of chronic myeloid leukemia (CML) cells harboring BCR-ABL kinase domain point mutations. SRC-family protein-tyrosine kinases interact with variety of cell-surface receptors and participate in intracellular signal transduction pathways; tumorigenic forms can occur through altered regulation or expression of the endogenous protein and by way of virally-encoded kinase genes. Dasatinib [2] is indicated for the treatment of newly diagnosed adults with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) in chronic phase, as well as adults with chronic, accelerated, or myeloid or lymphoid blast phase Ph+ CML with resistance or intolerance to prior therapy including imatinib, and adults with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) with resistance or intolerance to prior therapy. Dasatinib is also indicated for the treatment of paediatric patients 1 year of age and older with Ph+ CML in chronic phase or newly diagnosed Ph+ ALL in combination with chemotherapy. Dasatinib is an orally available small-molecule multikinase inhibitor. During clinical trials, less than 1% of patients treated with Dasatinib [3] had QTc prolongation as an adverse reaction, and 1% experienced a QTcF higher than 500 ms. The use of Dasatinib is also associated with myelosuppression, bleeding-related events, fluid retention, cardiovascular toxicity, pulmonary arterial hypertension, severe dermatologic reactions, tumor lysis syndrome and hepatotoxicity. It may also cause embryo-fetal toxicity and lead to adverse reactions associated with bone growth and development in paediatric patients.

The IUPAC Name of Dasatinib is N-(2-chloro-6-methyl phenyl)-2-[[6-[4-(2-hydroxy ethyl) piperazin-1-yl]-2-methyl pyrimidin-4-yl] amino]-1, 3-thiazole-5-carboxamide. The Chemical Structure of Dasatinib is following.

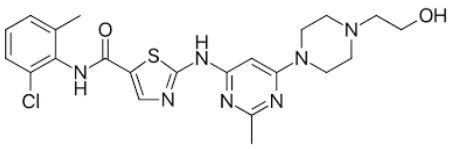

Fig 1: Chemical Structure of Dasatinib

Several analytical methods [33-36] have been devised for the determination of Dasatinib. These methods include titrimetric method, HPLC methods, HPTLC methods, LC methods, Uv- visible spectrophotometric methods, Amperometric method, fluorimetric method, Chemilumniscence method and voltametric methods.

These methods are required expensive or sophisticated instruments and not simple for routine analysis. High performance liquid chromatography [4, 5] (HPLC) can be used for determination of drugs and for purposes of control throughout the entire manufacturing process of drugs, as well as quality control of the finished product. It has the advantages of being accurate, sensitive, rapid, selective, and reproducible. The present paper reports the development of a new high performance liquid chromatography (HPLC) method for determination of Dasatinib in different type of pharmaceutical formulations.

Materials and Methods

Instruments Used

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Chemicals / Reagents Used

Table 2: List of Chemicals used

S.No.	Chemical Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development

Selection of Wavelength:

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent [6]. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Dasatinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Dasatinib. The scanned UV spectrum is attached in the following page,

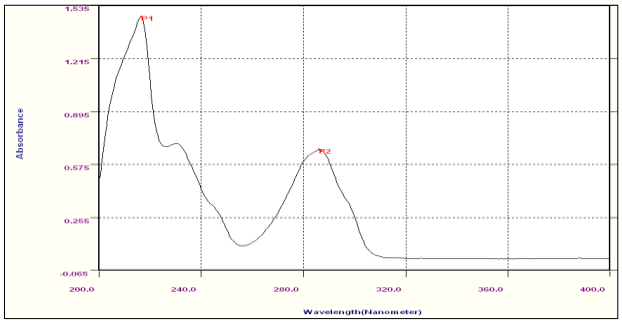

Fig 2: UV spectrum for Dasatinib

Sample & Standard Preparation for the UV-Spectrophotometer Analysis: 25 mg of Dasatinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase [7]. Further dilution was done by transferring 1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Observation: While scanning the Dasatinib solution we observed the maxima at 286nm. The UV spectrum [8] has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

Optimization of Chromatographic Conditions

The chromatographic conditions [9] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Xterra ODS (C_{18}) RP Column, 150 mm x 4.6 mm, 5µm	Methanol : Acetonitrile: Water = 40:30:30	0.8ml/min	286nm	Very Low response	Method rejected
Zorbax ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Methanol : Acetonitrile = 70 : 30	0.9ml/min	286nm	Low response	Method rejected
Develosil ODS (C_{18}) RP Column, 150 mm x 4.6 mm, 5µm	Acetonitrile: Methanol = 60 : 40	1.0ml/min	286nm	Tailing peaks	Method rejected
Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer (pH-5.2) : Methanol = 80:20	1.0ml/min	286nm	Resolution was not good	Method rejected
Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer (pH-3.8) : Methanol = 55:45	1.0ml/min	286nm	Tailing peak	Method rejected
Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer (pH-4.6) : Methanol = 65:35	1.0ml/min	286nm	Nice peak	Method accepted

Summary of Optimized Chromatographic Conditions: The Optimum Chromatographic conditions obtained from experiments [10] can be summarized as below:

Table 4: Summary of Optimized Chromatographic Conditions

Mobile phase	Phosphate Buffer (pH-4.6) : Methanol = 65:35%
Column	Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	286 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic
Retention time	4.778 minutes

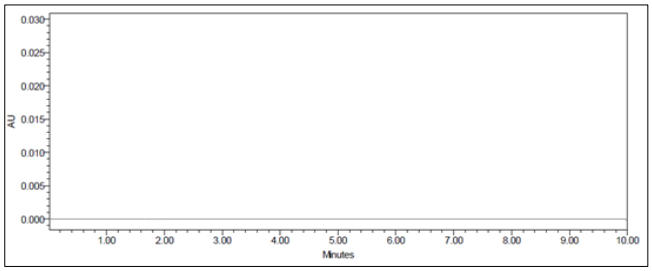

Fig 3: Chromatogram for Blank Solution

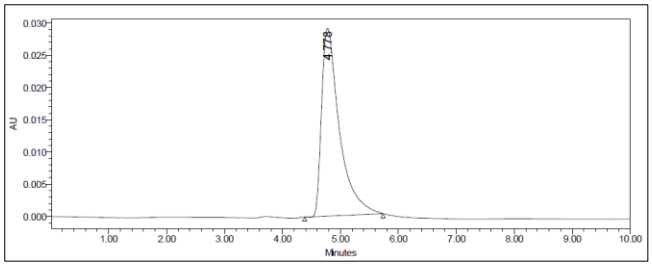

Fig 4: Chromatogram of Dasatinib in Optimized Condition

Preparation of Mobile Phase: 650ml of prepared phosphate buffer and 350ml of HPLC Grade Methanol were mixed well and degassed [11] in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Observation: The selected and optimized mobile phase was Phosphate Buffer (pH-4.6): Methanol = 65:35% and conditions optimized were flow rate (1.0 ml/minute), wavelength (286nm), Run time [12] was 10 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Method Validation

1. System Suitability Test

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test parameters [13-16] were established. The data are shown in Table-5.

Table 5: Data of System Suitability Test

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	4.817	745236	6986	1.39
2	Injection 2	4.783	743652	6857	1.37
3	Injection 3	4.840	742587	6856	1.36
4	Injection 4	4.783	742946	6847	1.39
5	Injection 5	4.817	743654	6896	1.38
6	Injection 6	4.778	741698	6874	1.37
Mean			**743295.5**	**6886**	**1.37666**
S.D			**1199.773604**		
%RSD			**0.161412736**		

Table 6: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Retention Time	RT > 2	Dasatinib= 4.778
2	Asymmetry	T ≤ 2	Dasatinib= 1.35
3	Theoretical plate	N > 2000	Dasatinib= 6859
4	Tailing Factor	T<2	Dasatinib= 1.37

2. Linearity:

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration [17] ranging from 60-140µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve [17] was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

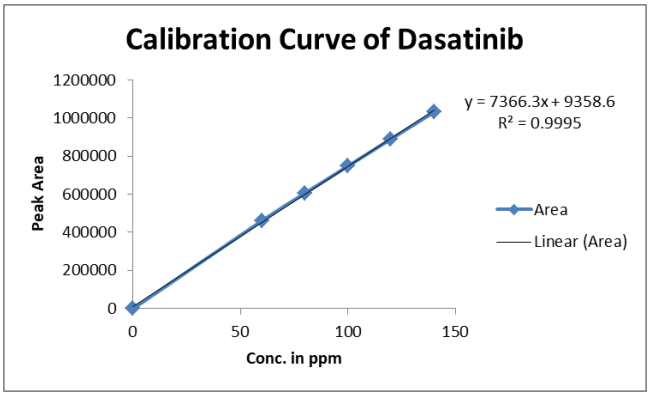

Fig 5: Calibration Curve of Dasatinib

Table 7: Linearity Data for Dasatinib

Conc. (µg/ml)	Area
0	0
60	461404
80	606157
100	748506
120	891041
140	1032196

3. Accuracy: The accuracy of the method was determined by recovery studies[18] and the percentage recovery [19] was calculated. The recoveries of Dasatinib were found to be in the range of 99-102%. The proposed Liquid Chromatographic method was applied to the determination of Dasatinib. The results for Dasatinib comparable with the corresponding labeled amounts.

Table 8: Shown Accuracy Observation of Dasatinib

Accuracy	Amount Added	Amount Recovered	Peak Area	% Recovery	Mean Recovery
80%	80	80.798	604517	100.997	100.437%
	80	80.673	603598	100.841	
	80	80.756	604213	100.945	
100%	100	99.933	745471	99.933	
	100	100.083	746574	100.083	
	100	100.365	748652	100.365	
120%	120	120.290	895415	100.241	
	120	120.201	894762	100.167	
	120	120.442	896541	100.368	

4. Precision

Repeatability: The precision [20] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Dasatinib (API). The percent relative standard deviation [21-23] was calculated for Dasatinib are presented in the table-9.

Table 9: Repeatability Data for Dasatinib

S. No.	Injection	Peak Area
1	Injection 1	743826
2	Injection 2	745277
3	Injection 3	742506
4	Injection 4	747576
5	Injection 5	746715
6	Injection 6	741278
7	**Average**	**744529.6667**
8	**SD**	**2440.4116**
9	**% RSD**	**0.32777**

Intermediate Precision

The Intermediate Precision [24] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table 10: Results of Intra-Assay & Inter-Assay

Conc. of Dasatinib (API) (µg/ml)	Observed Conc. of Dasatinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
80	80.096	0.487	79.685	0.688
100	100.074	0.968	100.057	0.789
120	120.056	0.847	120.016	0.698

Observations: The intra & inter day variation [25] of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Dasatinib revealed that the proposed method is precise.

5. Specificity

Specificity[26] can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared

individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.
The chromatograms representing the peaks of blank, Dasatinib and the sample containing the one drug was shown in following figures respectively.

Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

6. Limit of Detection (LOD) and Limit of Quantification (LOQ)
The LOD[27] and LOQ parameter was evaluated by mistreatment the slope of line and variance obtained from accuracy studies.
The detection limit (LOD) and quantization limit [28] (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).
L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response
S = Slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.
The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.469 & 4.454µg/ml respectively.

7. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate 1.0 ml (± 0.1ml/min), Wavelength of detection 286 (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness [29] of the method are also in favour of (Table-11, % RSD < 2%) the developed RP-HPLC method for the analysis of Dasatinib (API).

Effect of Variation of Flow Conditions
The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition
The sample was analyzed by variation of mobile phase [30] i.e. Methanol: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead of 35:65, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Table 11: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	742946	4.778	1.37	2896
Less Flow rate of 0.9 mL/min	698965	4.783	1.39	2986
More Flow rate of 1.1 mL/min	786598	4.817	1.42	2985
Less organic phase	732642	4.842	1.29	3102
More organic phase	702546	4.773	1.37	3247

8. Estimation of Dasatinib in Pharmaceutical Dosage Form
Label Claim: 100mg
Each tablet contains: 100mg
Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system [31] and the observations were recorded. The Assay [32] data are shown in Table-12.

Assay

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg Wt.} = \text{mg}$$

Where:
AT = Peak Area of drug obtained with test preparation
AS = Peak Area of drug obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Table 12: Recovery Data for estimation Dasatinib in Invista-100 Tablet

Brand Names of Dasatinib	Labelled Amount of Drug (mg)	Mean (± SD) Amount (mg) Found by the Proposed Method (n=6)	Assay % (± SD)
Invista-100 Tablet (Dr. Reddy's Laboratories)	100mg	99.596 (± 0.638)	99.428 (± 0.367)

Result & Discussion: The amount of drug in Invista-100 Tablet was found to be 99.596 (± 0.638) mg/tab for Dasatinib & % Purity was 99.428%.

Summary
To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Dasatinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case

of RP-HPLC various columns are available, but here Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm Column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Dasatinib it is evident that most of the HPLC work can be accomplished in the wavelength range of 286 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay and stability related impurity studies which can help in the analysis of Dasatinib in different formulations.

Conclusion

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Dasatinib in bulk and pharmaceutical dosage form. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Dasatinib indicated that the developed method is specific for the simultaneous estimation of Dasatinib in the bulk and pharmaceutical dosage forms.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The specific Retention time for Dasatinib are found to be 4.778min. The tailing factor was found to be 1.37 with theoretical plates 6859 for Dasatinib. The % Recoveries was determined as 100.437% for Dasatinib in Accuracy. The %RSD in Repeatability is 0.327 with Intermediate Precision (Intra & Inter Day) are 0.767 & 0.725 for Dasatinib in Precision respectively. In Linearity, the correlation coefficient was found to be 0.9995 for Dasatinib. The LOD for Dasatinib was 1.469 and LOQ for Dasatinib are 4.454 respectively.

References

1. DrugBank. Dasatinib. Available from: https://go.drugbank.com/drugs/DB01254
2. PubChem. Dasatinib. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Dasatinib
3. Wikipedia. Dasatinib. Available from: https://en.wikipedia.org/wiki/Dasatinib
4. Skoog DA, Holler FJ, Nieman TA. Principles of Instrumental Analysis. 5th ed. Harcourt Publishes Int Company; Chapter 28, p. 726-766.
5. Neue UD. HPLC Columns: Theory, Technology and Practice. Wiley-VC.
6. Katz E, Eksteen R, Schoenmakers P, Miller N. Handbook of HPLC. Vol. 78. Marcel Dekker Inc.
7. Ewing GW. Instrumental Methods of Chemical Analysis. 5th ed. McGraw Hill Book Company; 1988.
8. Fong GW, Long PL. HPLC in Pharmaceutical Industry. Marcel Dekker Series.
9. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. Himalaya Publishing House; p. 615-623.
10. Kealey D, Haines PJ. Analytical Chemistry. 1st ed. BIOS Scientific Publishers; 2002. p. 1-7.
11. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. CBS Publishers and Distributors; p. 157-174.
12. Government of India, Ministry of Health and Family Welfare. Indian Pharmacopoeia. Vol. 2. Delhi: Publication by Controller of Publication, 2007, pp. 484–554.
13. British Pharmacopoeia. International Ed. Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act 1968; 1993.
14. United States Pharmacopoeia 29 NF 24. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act; p. 587.
15. Skoog DA, West DM, Holler FJ, Crouch SR. Fundamentals of Analytical Chemistry. 8th ed. Cengage Learning India Pvt Ltd; 2009. p. 271-280.
16. Kasture AV, Mahadik KR, Wadodkar SG, More HN. A Textbook of Pharmaceutical Analysis, Instrumental Methods. 9th ed. Nirali Prakashan; vol. 2, p. 5-7, 28-30.
17. Settle FA. Handbook of Instrumental Techniques for Analytical Chemistry. 1st ed. Singapore: Pearson Education Inc.; 2004.
18. Willard HH, Dean AJ. Instrumental Methods of Analysis. 7th ed. CBS Publishers and Distributors; 1986. p. 513-515.
19. Connors KA. A Textbook of Pharmaceutical Analysis. 3rd ed. Wiley Interscience Publication; 2005. p. 373-400.
20. Ahuja S. High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications; 2006.
21. Amesham Biosciences. Principles and Methods of Reversed Phase Chromatography. p. 6-8.
22. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. John Wiley and Sons Inc.; 1997.
23. Mohammad T, Pandya K, Zaveri M, Sathe P. HPLC Method Development and Validation for Pharmaceutical Analysis: A Review. Int Pharmaceutica Sciencia. 2012; 2(3):14.
24. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. Wiley; 2001.
25. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method: A review. Int Res J Pharm Appl Sci. 2012; 2(4):22-23.
26. Bliesner DM. Validating Chromatographic Methods. John Wiley & Sons Inc.; 2006. p. 88-92.
27. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Methodology. ICH Guidelines Q2B; 1996. p. 11. (CPMP/ICH/281/95).
28. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method: A review. Int Res J Pharm Appl Sci. 2012; 2(4):17-25.
29. Bhardwaj SK, Dwivedi K, Agarwal D, Pathak N, Agarwal D. A Review: HPLC Method Development and Validation. Int J Anal Bioanal Chem. 2015.
30. Chromacademy. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC.
31. Sonawane LV. Bioanalytical Method Validation and Its Pharmaceutical Application: A Review. Pharm Anal Acta. 2014; 5(3).
32. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Text and Methodology. ICH Guidelines Q2 (R1).
33. Sankar PR, Anusha S. Development and Validation of RP-HPLC Method for the Determination of Dasatinib in Tablet Dosage Form. Int J Pharm Sci Res. 2019; 10(10):4531-4537.
34. Ramachandra B, Naidu NV. Validation of RP-HPLC

Method for Estimation of Dasatinib in Bulk and Its Pharmaceutical Dosage Forms. Int J Pharm Biol Sci. 2014; 4(1):61-68.
35. Jogarao YSRV, Bharath P, Siva Ramakrishna V, Ramachandran D. Development & Validation of RP-HPLC Method for Quantitative Estimation of Dasatinib and Its Impurities in Pharmaceutical Dosage Form. J Pharm Res Int. 2022; 34(25A):52-65. DOI: 10.9734/jpri/2022/v34i25A35950.
36. Nataraj KS, Sivalingachari P, Sai Naveen K, Alekhya K. Method Development and Validation for the Estimation of Dasatinib Monohydrate Tablets by RP-HPLC. Asian J Res Chem. 2013; 6(9):859-862.