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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF DASATINIB IN TABLET DOSAGE FORM

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Keywords:

Dasatinib, RP-HPLC, Validation, ICH guidelines

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ABSTRACT: The objective of the present study was to develop and validate a novel RP-HPLC method for the determination of Dasatinib in the pharmaceutical dosage form. Chromatographic separation was conducted on agilent technologies-1260 series with the G1311C quaternary pump, Thermo Scientific C_{18} column (4.6 mm i.d. × 250 mm, 5 µm particle size) and equipped with photodiode array detector G1315D. The mobile phase consisted of methanol and acetonitrile mixed in the ratio of 50:50 v/v, was used at a flow rate of 1 ml/min, and the detection wavelength was set at 323 nm. The retention time for Dasatinib was found to be 4.073 min. The calibration was linear (r^2 = 0.999) in the concentration range of 2-10 µg/ml. The limit of detection and the limit of quantitation were found to be 0.5263 µg/ml and 1.5948 µg/ml, respectively. Recovery of Dasatinib in tablet formulation was observed in the range of 98.09-99.57%. Percentage assay of Dasatinib (Dasanat) was found to be 99.45% w/w. Thus the novel proposed method for Dasatinib was found to be feasible for the estimation of Dasatinib in bulk as well as the pharmaceutical dosage form.

INTRODUCTION: The chemical name of Dasatinib is N-[2-Chloro-6-methylphenyl] -2- [[6-[4-(2-hydroxyethyl) -1- piperazinyl] -2-methyl-4-pyrimidinyl] amino] -5-thiazole carboxamide **Fig 1**. Dasatinib is utilized for the treatment of chronic myeloid leukemia and acute lymphoblastic leukemia. A thorough literature survey of Dasatinib revealed that very few analytical methods had been reported for estimation of Dasatinib hitherto. Majority of methods for determination of Dasatinib in biological fluids and pharmaceutical dosage forms includes LC-MS/MS [1-4], LC-MS [5-6], HPTLC-LC [7], HPTLC [8], UPLC-MS [9], HPLC-MS [10], RP-HPLC [11] and UV-Visible Spectrophotometric method [12-13].

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This novel proposed method contributes quick estimation, correct peak shape, precise, simple, and quick, use of smaller sample volumes and utilizing methanol as a mobile phase which is economical when compared with other existing methods. So, it is necessary to develop a simple, precise, and rapid RP-HPLC method for the quantitative determination of Dasatinib. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1) [14-18]. The chemical structure of dasatinib is shown in **Fig. 1**.

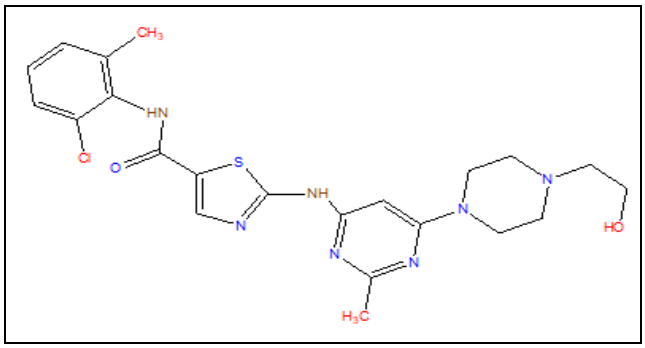

FIG. 1: CHEMICAL STRUCTURE OF DASATINIB

MATERIALS AND METHODS:

Chemicals and Reagents: The above said standard drug was gifted from Hetero Labs Ltd., Hyderabad, India. All the chemicals used in this method were of high-grade purity and purchased from Merck Chemical Division Ltd., Mumbai. HPLC grade acetonitrile, water, methanol, and triethylamine were obtained from Merck pharmaceuticals private Ltd., Mumbai, India. Commercial tablets of the above said formulation was obtained from a local pharmacy.

Instrumentation and Conditions: The High-Pressure Liquid Chromatographic system utilized was an Agilent high-pressure liquid chromatograph 1260 series with the GI311C quaternary pump, Thermo Scientific C_{18} column (5 µm particle size X 4.6 × 250 mm) (made in the USA) and a diode array detector G1315D was utilized. Ezchrome elite software was used for chromatography data acquisition, processing, and control of HPLC chromatograph. Digital pH meter (systronics model - 802), an electronic balance (Shimadzu TX223L), a sonicator (spectral lab, model UCB 40) and UV-Visible spectrophotometer (systronics model-2203) were used in this study.

Preparation of Mobile Phase: To prepare mobile phase HPLC grade methanol and acetonitrile were mixed in the ratio of 50:50% v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Stock and Working Standard Solutions: Accurately 10 mg of pure Dasatinib was weighed and transferred into 10 ml clean volumetric flask and 5 ml mobile phase was added, if necessary, sonicate to dissolve. The volume was adjusted up to the mark with the mobile phase. This is the primary stock solution of Dasatinib with a concentration of 1000 µg/ml.

The secondary stock solution is prepared by adding 1 ml of primary stock solution in 10 ml volumetric flask and made up the volume with a mobile phase having the concentration range 100 µg/ml. Five standard working solutions were prepared for the calibration graph by adding defined volumes of the secondary stock solution and diluting with the mobile phase. The concentrations of Dasatinib are 2, 4, 6, 8, and 10 µg/ml, respectively.

Sample Preparation for Tablets: Accurately weighed twenty Dasatinib tablets, and the average weight was calculated. Accurately weighed a portion of tablet powder equivalent to 100 mg of Dasatinib and transferred into a 100 ml volumetric flask to this 50 ml mobile phase was added and sonicated for 15 min. The mobile phase was adjusted up to the mark. The solution was filtered using a 0.45 µm nylon filter. From the above solution pipette out 1.0 ml into a 100 ml volumetric flask and dilute with mobile phase up to the mark and mix well. It was further diluted to get the desired concentration. The amount present in the tablet was calculated from the plotted calibration graph or utilizing the regression equation. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20 µl fixed sample loop of the injection port.

Analytical Method Validation: Once the chromatographic and the experimental conditions were established, the method was validated by the determination of the following parameters such as specificity, system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH Q2 (R1) guidelines.

System Suitability Parameters: The chromatographic systems used for analysis must pass system suitability before going to start the experiment. At first HPLC system is stabilized for forty minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters such as tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and retention time. The % RSD for the peak area of six replicate injections of Dasatinib standard NMT 2.0. The parameters, such as tailing factor, % RSD, and theoretical plates, were studied.

Linearity: Standard stock solution of the Dasatinib (1 mg/ml) was prepared with the mobile phase. To study the linearity range of drugs, serial dilutions were made from a standard stock solution in the range of 2-10 µg/ml.

Specificity: Specificity of an analytical method is its ability to measure accurately and specifically the

analyte of interest without interference from placebo and degradation products. The specificity of the method was established by injecting blank, placeb, and standard solution in triplicate and recording the chromatograms.

Precision: The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by performing six repeated analysis of the same working solution of Dasatinib on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on different days and also by another analyst performing the analysis in the same laboratory (between-analysts).

Accuracy: The accuracy of a method is defined as the closeness of a measured value to the true value. The recovery studies were carried out at 50%, 100% and 150% of the target level in the tablet in triplicate each in the presence of placebo.

Robustness: The robustness was determined by analyzing the same sample under various conditions. The factors considered to be: variations in the flow rate, the organic ratio of the mobile phase, and pH. There were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing that the method is robust. The % RSD of Dasatinib should not be more than 2.0%.

LOD and LOQ: Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated based on using following formulas, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is the deviation response, S is the slope of the calibration curve.

RESULTS AND DISCUSSION:

Method Development and Optimization: The current study was aimed at developing a sensitive, rapid, and accurate reversed-phase HPLC method for the analysis of Dasatinib in bulk drug and the pharmaceutical dosage form. To get decorous retention time, sharp and well-resolved peaks, the parameters such as different flow rates, detection wavelength, a choice of mobile phases containing acetonitrile, methanol, and HPLC grade water were studied. Good quality symmetrical sharp peak, minimum tailing factor in short run time was obtained with C_{18} column and mobile phase composed of methanol: acetonitrile in the ratio of 50:50 v/v, at a flow rate of 1 ml/min with maximum λ_{max} at 323 nm. All the system suitability parameters were computed at the optimized chromatographic conditions. The retention time of 4.073, plate number of 8762, and a tailing factor of 1.2021 were obtained for Dasatinib. The obtained values of the entire system suitability parameters are within the limits of the agreeable range, which shows that the proposed method is fit for the detection of Dasatinib in the tablet form. The optimum chromatographic conditions and system suitability parameters are tabulated in **Table 1**.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS AND SST

Parameter	Chromatographic conditions
Instrument	Agilent Technologies 1260 series with the G1311C quaternary pump
Column	Thermo Scientific C_{18} column (4.6 mm i.d. × 250 mm, 5 µm particle size)
Detector	1260 series DAD VL photo diode array detector G1315D
Mobile phase	Methanol: acetonitrile (50:50% v/v)
Flow rate	1 ml/min
Detection wavelength	UV at 323 nm
Run time	8 min
Temperature	Room temperature (25 °C)
Volume of injection loop	20 µl
Retention time*	4.073 min
Theoretical plates [th.pl]*	8762
Tailing factor*	1.2021

*Number of six determinations

Linearity: The calibration curve was constructed between concentrations versus peak area prepared in the concentration range of 2-10 µg/ml of stock solution. The results are tabulated in **Table 2**.

TABLE 2: CALIBRATION DATA OF DASATINIB

S. no.	Concentration (µg/ml)	Peak area, (mAU)
1	2	49801
2	4	94609
3	6	143681
4	8	196870
5	10	244838

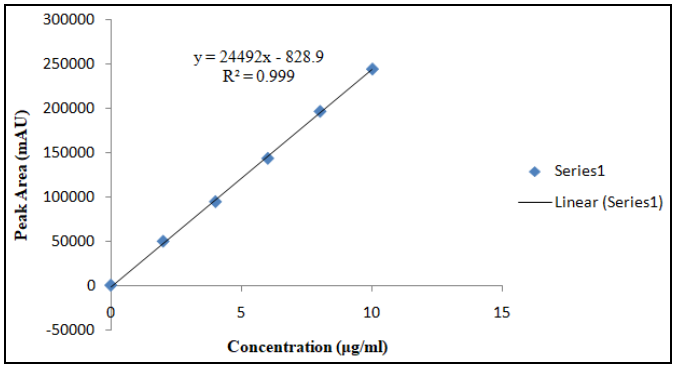

FIG. 2: CALIBRATION GRAPH OF DASATINIB

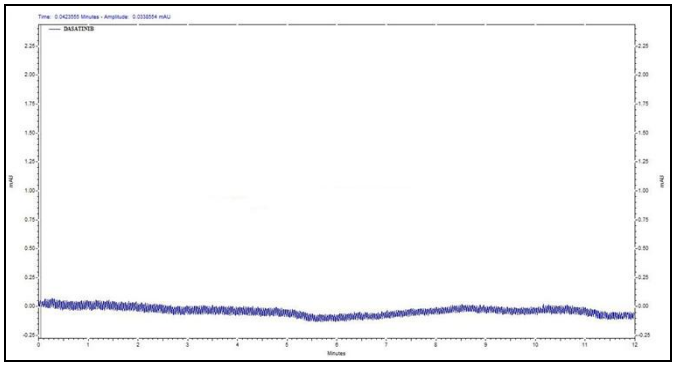

FIG. 3: BLANK CHROMATOGRAM OF DASATINIB

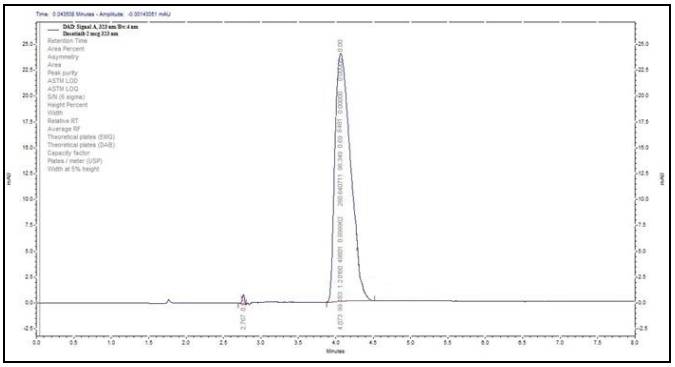

FIG. 4: STANDARD CHROMATOGRAM OF DASATINIB (2 µg/mL)

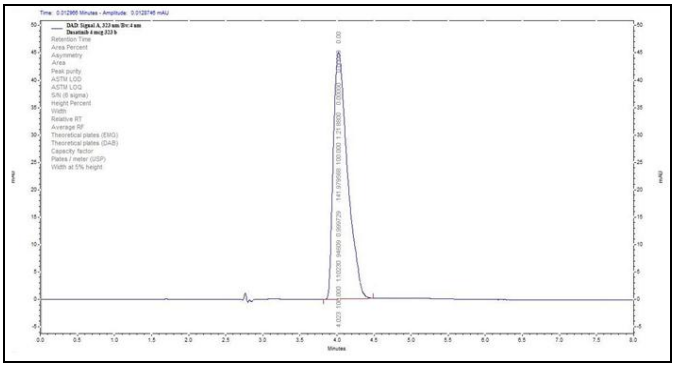

FIG. 5: STANDARD CHROMATOGRAM OF DASATINIB (4 µg/mL)

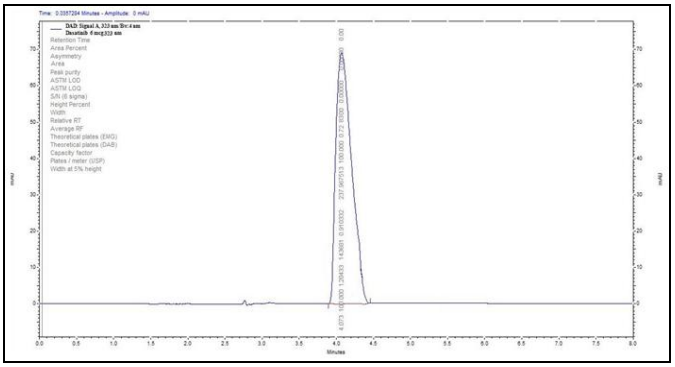

FIG. 6: STANDARD CHROMATOGRAM OF DASATINIB (6 µg/mL)

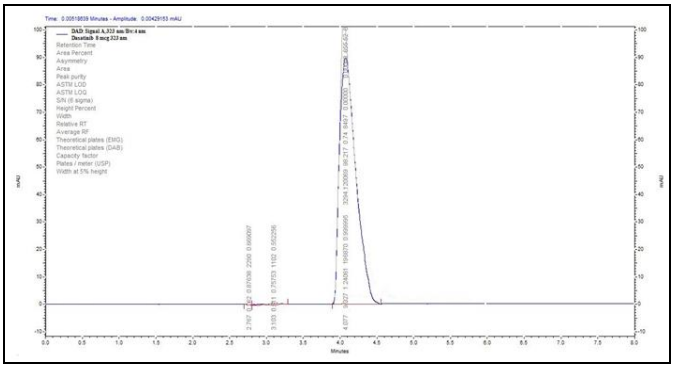

FIG. 7: STANDARD CHROMATOGRAM OF DASATINIB (8 µg/mL)

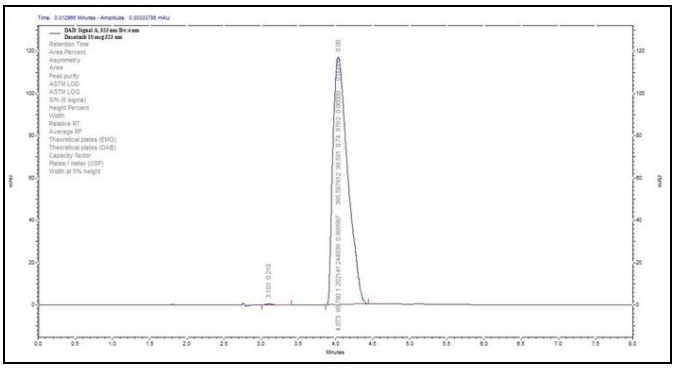

FIG. 8: STANDARD CHROMATOGRAM OF DASATINIB (10 µg/mL)

The calibration graph of Dasatinib is presented in **Fig. 2**. The regression equation was found to be Y = 24492x - 828.9. The correlation coefficient of Dasatinib r^2 was noted as 0.999, which states that the method was good, linear to the concentration versus peak area responses. The results show that a phenomenal relationship between peak area and concentration of the drug in the calibration curve.

The standard chromatograms of Dasatinib are presented in **Fig. 4 to 8**.

Specificity: Commonly used tablet excipients did not interfere with this method. It shows that the method is specific. Furthermore, the well-shaped peaks also indicate the specificity of the method. The specificity results are tabulated in **Table 3**.

TABLE 3: SPECIFICITY STUDY

Name of the solution	Retention time (t_R) min
Mobile phase	No peaks
Placebo	No peaks
Dasatinib 10 µg/ml	4.073 min

Precision: The system precision is done by using the standard chemical substance to ensure that whether the analytical system is working properly or not. The retention time and area of 6 determinations were measured, and RSD was calculated. Blank and Standard solutions were injected six times into the HPLC system, and the chromatograms were recorded to obtain RSD. System precision results are shown in **Table 4**. It was noted that the % RSD values of precision for intra-day and inter-day **Table 5** precision was 0.0022 and 0.0016, respectively. Intra-day and inter-day % RSD values lower than 2% assuring that this method was found to be fairly precise and reproducible.

TABLE 4: RESULTS OF SYSTEM PRECISION

Sample	Concentration (µg/mL)	Injection no.	Peak area (mAU)	Mean ± SD*	% RSD#
Dasatinib	10	1	244830	244814.2 ± 56.4815	0.023
		2	244827		
		3	244838		
		4	244841		
		5	244849		
		6	244700		

*Each value is represented as a mean ± SD of 6 observations (n=6), SD: Standard Deviation, RSD: Relative Standard Deviation, # Acceptance criteria <2.0.

TABLE 5: INTRA-DAY AND INTER-DAY PRECISION DATA OF DASATINIB

Concentration (µg/mL)	Intra-day precision	Inter-day precision		
		Day 1	Day 2	Day 3
6	143680	143682	143681	143679
	143683	143680	143684	143682
	143681	143684	143682	143680
	143685	143686	143680	143683
	143687	143681	143685	143684
	143688	143685	143679	143681
Mean ± SD*	1436 ± 3.224	1436 ± 2.366	1436 ± 2.316	1436 ± 1.870
% RSD#	0.0022	0.0016	0.0016	0.0013

*Each value is represented as a mean ± SD of 6 observations (n=6), SD: Standard Deviation, RSD: Relative Standard Deviation, # Acceptance criteria <2.0.

Accuracy: A study of recovery was conducted for Dasatinib intact tablet from about 50 %, 100 %, and 150 % of the initial assay concentration. Sample solutions were prepared in triplicate for each level and analyzed as per test method. The individual % recovery, % average recovery, and % RSD for recovery at each level were calculated, and the results are found to be within the limit. Accuracy results are shown in **Table 6**.

Robustness: The robustness of the developed method was evaluated by small, deliberate changes in method parameters such as flow rate (± 0.2 ml/min), detection wavelength (± 5 nm) and mobile phase composition (± 5 %). The % RSD values of robustness, which is less than 2% reveal that the proposed method is robust. The results of robustness study results are shown in **Table 7**.

TABLE 6: RESULTS OF ACCURACY

% Level Spiked	% Recovery	Mean % Recovery	% RSD
50	99.85	99.57	0.552
	98.94		
	99.93		
100	97.58	98.09	1.503
	99.76		
	96.95		
150	98.86	99.21	0.542
	99.83		
	98.94		

Acceptance Criteria: The Individual % recovery should be between 95.0 and 105.0. The average % recovery of each level should be between 97.0 and 102.0 and % RSD for recovery at each level should not be more than ± 5.

TABLE 7: ROBUSTNESS RESULTS OF DASATINIB

Parameters	Optimized	Used	Retention time (min)	Plate count $	Peak asymmetry #	Remarks
Flow rate	1.0 ml/min	0.8 ml/min	3.997	8780	1.12	*Robust
(± 0.2 ml/min)		1.0 ml/min	4.073	8762	1.20	*Robust
		1.2 ml/min	4.211	8750	1.23	*Robust
Detection wavelength	323 nm	318 nm	4.073	8762	1.25	Robust
(± 5 nm)		323 nm	4.073	8762	1.20	Robust
		328 nm	4.073	8762	1.23	Robust
Mobile phase composition	50:50 v/v	45:55 v/v	4.011	8750	1.20	*Robust
(MEOH:ACN)		50:50 v/v	4.073	8762	1.20	*Robust
		55:45 v/v	4.019	8769	1.22	*Robust

Acceptance criteria (Limits): # Peak Asymmetry < 1.5, $ Plate count > 2000, * Significant change in retention time.

LOD and LOQ: The developed method was found to be highly sensitive with LOD of 0.5263 µg/ml and LOQ of 1.5948 µg/ml. The LOD and LOQ values are presented in **Table 8**. The results of LOD and LOQ supported the sensitivity of the developed method.

TABLE 8: LOD AND LOQ RESULTS OF DASATINIB

Limit of detection (LOD)	0.5263 µg/ml
Limit of quantitation (LOQ)	1.5948 µg/ml

Analysis of Tablet Formulation: The developed and validated method was successfully applied for the determination of Dasatinib in their tablet dosage form. The assay result **Table 9** shows that the amount of the drug was in excellent agreement with the labeled value of the formulation. The representative sample chromatogram of Dasatinib is shown in **Fig 9**. **Table 10** represents the summary of validation parameters.

TABLE 9: RESULTS OF ANALYSIS OF DASATINIB

S. no.	Formulation	Labelled amount mg/tablet	Amount found mg/tablet	Mean % Assay ± SD	% RSD*
1	Dasanat tablets	20	19.89	99.45 ± 1	1.005

*Average of six determinations; SD: standard deviation; RSD: relative standard deviation

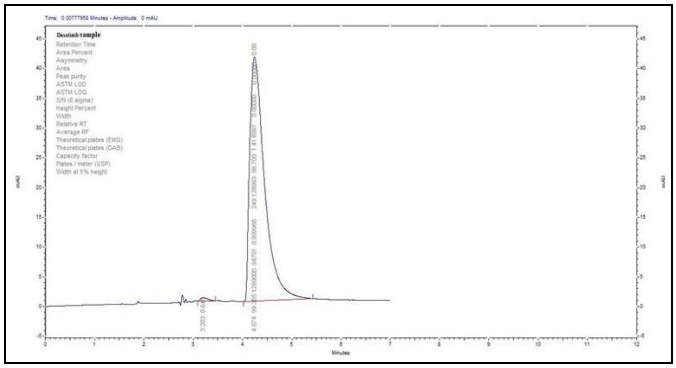

FIG. 9: DASATINIB SAMPLE CHROMATOGRAM

TABLE 10: SUMMARY OF VALIDATION PARAMETERS

Validation Parameters	Results
Detection wavelength (λ_{max})	323 nm
Beer's law limits (µg/ml)	2-10 µg/ml
Regression equation	Y = 24492x - 828.9
Correlation coefficient (r^2)	0.999
Flow rate	1 ml/min
Retention time (R_t)	4.073 min
Intra-day Precision (% RSD)	0.0022
Inter-day Precision (% RSD)	0.0016
Accuracy (% recovery)	98.09 - 99.57% w/w
Limit of Detection (µg/ml)	0.5263 µg/ml
Limit of Quantitation (µg/ml)	1.5948 µg/ml
Assay (% w/w)	99.45% w/w

CONCLUSION: In conclusion, a simple, accurate, sensitive, rapid, and precise RP-HPLC method was developed and validated for the estimation of Dasatinib in the pharmaceutical dosage form. Statistical analysis for the above said results demonstrates that the method is fit for the estimation of Dasatinib in tablet forms. The assay values were in good agreement with their respective labeled claim. The absence of interfering peaks in the chromatogram suggests that the tablet excipients do not interfere with the estimation of the drug by the proposed method. Hence, it is concluded that the proposed method can be utilized for research studies, quality control, and routine analysis for the quantification of Dasatinib in tablet dosage form with lesser resources available.

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CONFLICT OF INTEREST: Nil

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