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Development and Validation of RP-HPLC Method for Estimation of Dasatinib in bulk and its Pharmaceutical formulation

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Dasatinib in Bulk and its pharmaceutical formulation. Separation was achieved with a Cosmicsil BDS C18 ((Make: Nomura chemicals (Japan); 150 x 4.6mm I.D; particle size 5 µm)) Column and Triethlyamine buffer (pH adjusted to 6.5 ± 0.05 with diluted orthophosphoric acid): Maethanol and Acetonitrile (50:50) v/v as eluent at flow rate 1.0 mL/min and the Column temperature was 35°C. UV detection was performed at 315 nm and sample temperature was maintained at 5°C. The method is simple, rapid, and selective. The described method of Dasatinib is linear over a range of 3.821 µg/mL to 57.314 µg/mL. The method precision for the determination of assay was below 2.0% RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 98.5 to 99.8 %. The method enables accurate, precise, and rapid analysis of Dasatinib. It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

Keywords: RP-HPLC**,** Dasatinib, tyrosine kinases

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INTRODUCTION

Dasatinib is an inhibitor [1,2] of multiple tyrosine kinases. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. 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[2]. The molecular formula is $C_{22}H_{26}C_{l}N_7O_2S.H_2O$, 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 available in market with different strengths like 20 mg, 50 mg, 70 mg, 80 mg, 100 mg, and 140 mg. Literature survey reveals that there is no RP-HPLC methods reported for quantification of dasatinib in pharmaceutical formulation[4-5]. However, a few methods have been used for quantification of major tyrosine kinase inhibitors, imatinib, dasatinib, and nilotinib, in human plasma[5]. Quantification with HPLC-mass spectrometry has been reported[5]. Therefore, the present study aims to develop and validate a simple, fast and economical RP-HPLC method for estimation of dasatinib in bulk and its pharmaceutical dosage forms.

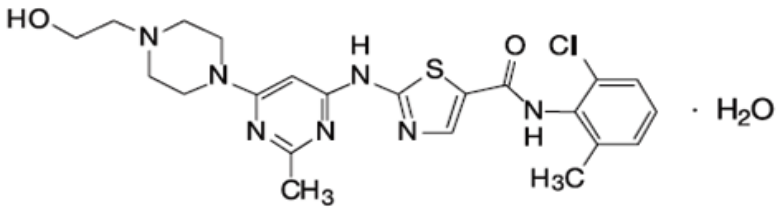

Figure 1: Chemical Structure of Dasatinib

MATERIALS AND METHODS

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Cosmicsil BDS C18 ((Make: Nomura chemicals (Japan); 150 x 4.6mm I.D; particle size 5 µm)) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Chemicals and Solvents

HPLC Grade water (Millipore), Acetonitrile (HPLC Grade), Triethyl amine (HPLC Grade), Orthophosphoric acid (HPLC Grade), Methanol (GR Grade) and Sodium dihydrogen phosphate monohydrate of GR Grade were obtained from E. Merck (India) Ltd., Mumbai.

a. Buffer Preparation

Add 4.0mL of Triethlyamine to 1000mL water and adjust the pH to 6.5 ± 0.05 with diluted orthophosphoric acid. Then add 10mL of methanol and mix well.

b. Solvent Mixture

Prepare a mixture of Methanol and Acetonitrile in the ratio of 50:50 v/v respectively.

c. Mobile Phase:

Prepare a filtered and degassed mixture of Buffer preparation and solvent mixture in the ratio of 50:50 v/v respectively.

d. Diluent:

Mobile phase.

e. Standard Preparation

Accurately weigh and transfer about 50.0 mg of Dasatinib monohydrate working standard in to 100 mL volumetric flask. Add about 60 mL of solvent mixture, sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 2.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

f. Sample Preparation

Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 100.0 mg of Dasatinib into a 250 mL volumetric flask. Add about 160 mL of solvent mixture, shake on orbital shaker for 15 minutes and sonicate for 30 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with solvent mixture. Centrifuge the solution at 3000 RPM for 15 minutes. Transfer 5.0 mL of the above solution into a 100 mL volumetric flask, dilute to volume with diluent.

g. System Suitability

Chromatograph the standard preparation (six replicate injections) and measure the peak area responses for the analyte peak and evaluate the system suitability parameters as directed.

Procedure

Separately inject equal volumes (10 µL) of diluent, standard preparation and sample preparations into the chromatograph, record the chromatograms, and measure the peak area responses for the major peaks. Calculate the quantity in % of Dasatinib in the portion of Dasatinib tablets taken by using the following formula.

% Of Labeled amount of Dasatinib:

$$\frac{TA}{SA} \times \frac{SW}{100} \times \frac{2}{50} \times \frac{250}{TW} \times \frac{100}{5} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{LA} \times 100$$

Where,

TA : Peak area response due to Dasatinib from sample preparation

SA : Peak area response due to Dasatinib from standard preparation.

SW : Weight of Dasatinib monohydrate working standard, taken in mg.

P : % Assay of Dasatinib monohydrate working standard, taken on as is basis.

Avg. Wt : Average weight of tablet in mg.

LA : Label Amount of Dasatinib, in mg.

Chromatographic Conditions

A Cosmicsil BDS C18 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5µm)) Column was used for analysis at column temperature 35°C. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL and the sample temperature was maintained at 5°C. The photodiode array detector was set to a wavelength of 315 nm for the detection and Chromatographic runtime was 12 minutes.

Note: For Needle wash use Mobile phase.

RESULTS AND DISCUSSION

Method Development

To develop a suitable and robust HPLC method for the determination of Dasatinib, different mobile phases were employed to achieve the best separation and resolution. The method development was started with a Cosmicsil BDS C18 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 µm)) with the following mobile phase. Add 4.0mL of Triethlyamine to 1000mL water and adjust the pH to 6.5 ± 0.05 with diluted orthophosphoric acid. Then add 10mL of methanol and mix well. Filter the solution through 0.45µm membrane filter. Prepare a filtered and degassed mixture of Buffer and mixture of Methanol and Acetonitrile in the ratio of 60:40 v/v respectively. Dasatinib peak was eluted at void volume. For next trial the mobile phase composition was changed slightly.

The mobile phase composition was Buffer, mixture of Methanol and Acetonitrile in the ratio of 60:40 v/v. In the above trail, the peak shape was broad. The mobile phase composition changed to respectively Buffer and mixture of Methanol and Acetonitrile in the ratio of 50:50 v/v respectively as eluent at flow rate 1.0 mL/min and the Column temperature was 35°C. UV detection was performed at 315 nm and the sample temperature was maintained at 5°C. The retention time of Dasatinib is 7.236 minutes as shown in Figure 5 and the peak shape was good. The chromatogram of Dasatinib standard using the proposed method is shown in Figure 4. System suitability results of the method are presented in Table 1. Dasatinib shows significant UV

absorbance at Wavelength 315 nm. Hence this wavelength has been chosen for detection in analysis of Dasatinib.

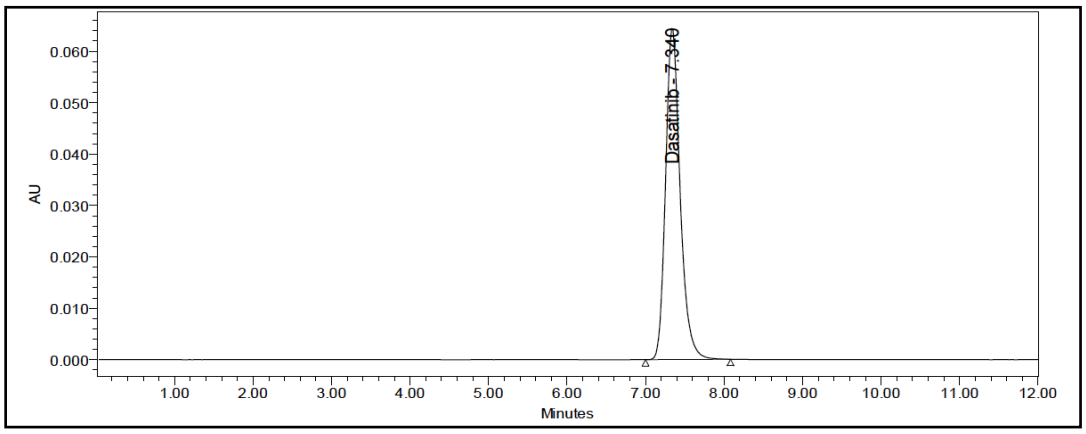

Figure 4: HPLC Chromatogram of Dasatinib Standard.

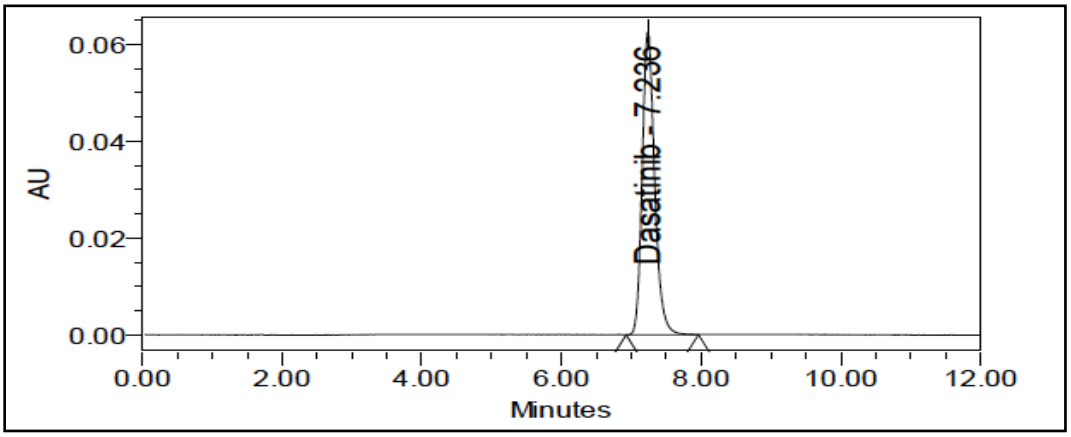

Figure 5: Typical HPLC Chromatogram of Dasatinib tablets 50mg.

Table 1: System Suitability Report

S. No	System suitability Parameters	Result	Acceptance Criteria
1.	%RSD for six replicate injections of Dasatinib peak area	0.5 %	NMT 2.0 %
2.	Tailing factor for Dasatinib peak	1.2	NMT 2.0
3.	Theoretical plate count for Dasatinib peak	8272	NLT 2000

NMT-Not More Than NLT-Not Less Than

Column Selection:

Based on the retention and better peak shape of the compound Cosmicsil BDS C18 5 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 µm)) Column was selected as a suitable column for analysis of Dasatinib.

Method Validation:

The developed LC method extensively validated for assay of Dasatinib using the following parameters.

Specificity (Blank and Placebo Interference)

A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of Blank and Placebo solutions showed no peaks at the retention time of Dasatinib peak. This indicates that the excipients used in the formulation do not interfere in estimation of Dasatinib in Dasatinib tablets. The chromatogram of Dasatinib Blank and Placebo using the proposed method is shown in Figure 2 and Figure 3.

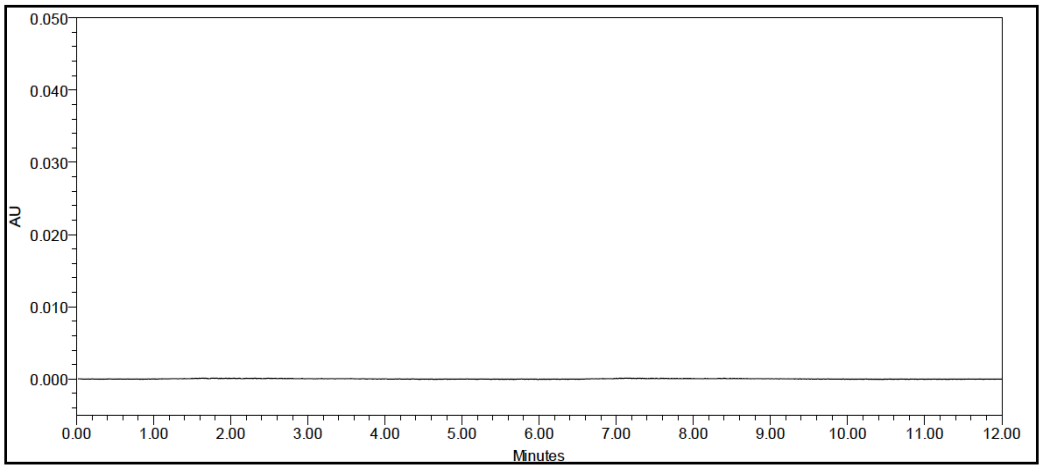

Figure 2: HPLC Chromatogram of Dasatinib Blank.

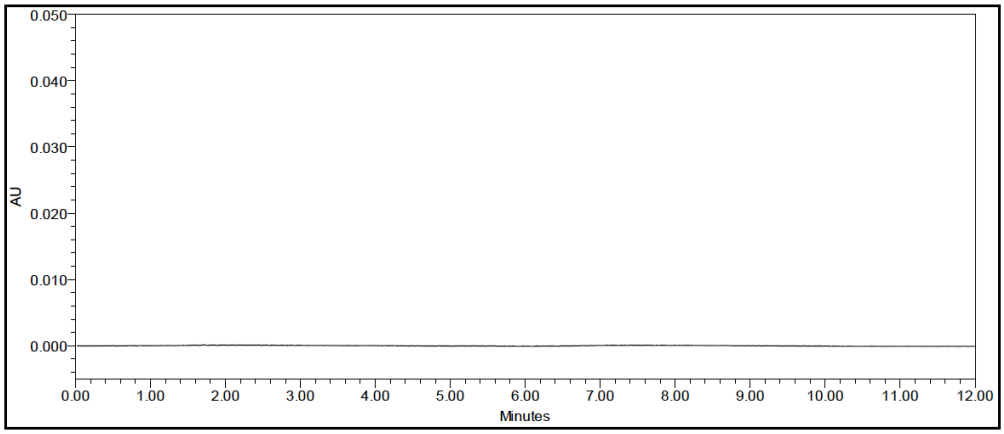

Figure 3: HPLC Chromatogram of Dasatinib Placebo.

Linearity

A study to establish the linearity of detector response of Dasatinib monohydrate was conducted from 20% (3.821 µg/mL) level to 300% (57.314 µg/mL) level of the Dasatinib monohydrate standard concentration. Plotted linearity graphs of Dasatinib standard concentration versus peak area of 20%, 50%, 80%, 90%, 100%, 110%, 120%, 150%, 200% & 300% level and has been found linear in the prescribed range. Linearity graph is shown in **Fig**ure 5. Linearity results of the method are presented in **Table** 2.

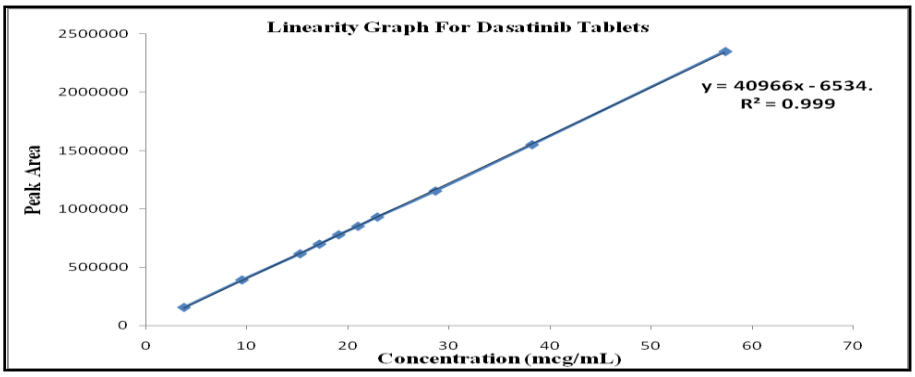

Figure 5: Linearity graph for Dasatinib.

Table 2: Linearity Table Report

% Level	Concentration (mcg/mL)	Peak Area
20	3.821	155829
50	9.552	391288
80	15.284	615931
90	17.194	698548
100	19.105	779121
110	21.015	852237
120	22.926	931356
150	28.657	1154891
200	38.209	1551923
300	57.314	2351817
Correlation Coefficient (r)		1.000
Regression Coefficient (r^2)		0.9999
Y-Intercept		-6534.3
Slope		40966.2
Residual sum of squares		417867989.7
Y-Intercept / response at 100 % of working concentration X 100		-0.8

Table 5: Results for Precision of Test Method

S. No	% Assay of Dasatinib present in tablets (50 mg)
1.	102.0
2.	102.0
3.	102.1
4.	101.9
5.	102.2
6.	101.4
Average	101.19
SD	0.28
% RSD	0.3

Precision of Test Method

The precision of test method was evaluated by analyzing six samples of Dasatinib in Dasatinib tablets, 50 mg as per test method. The % RSD of Assay of Dasatinib in Dasatinib tablets, 50mg

was found within the limits. The results were given in **Table 5**. A typical HPLC Chromatogram is shown in Figure 6.

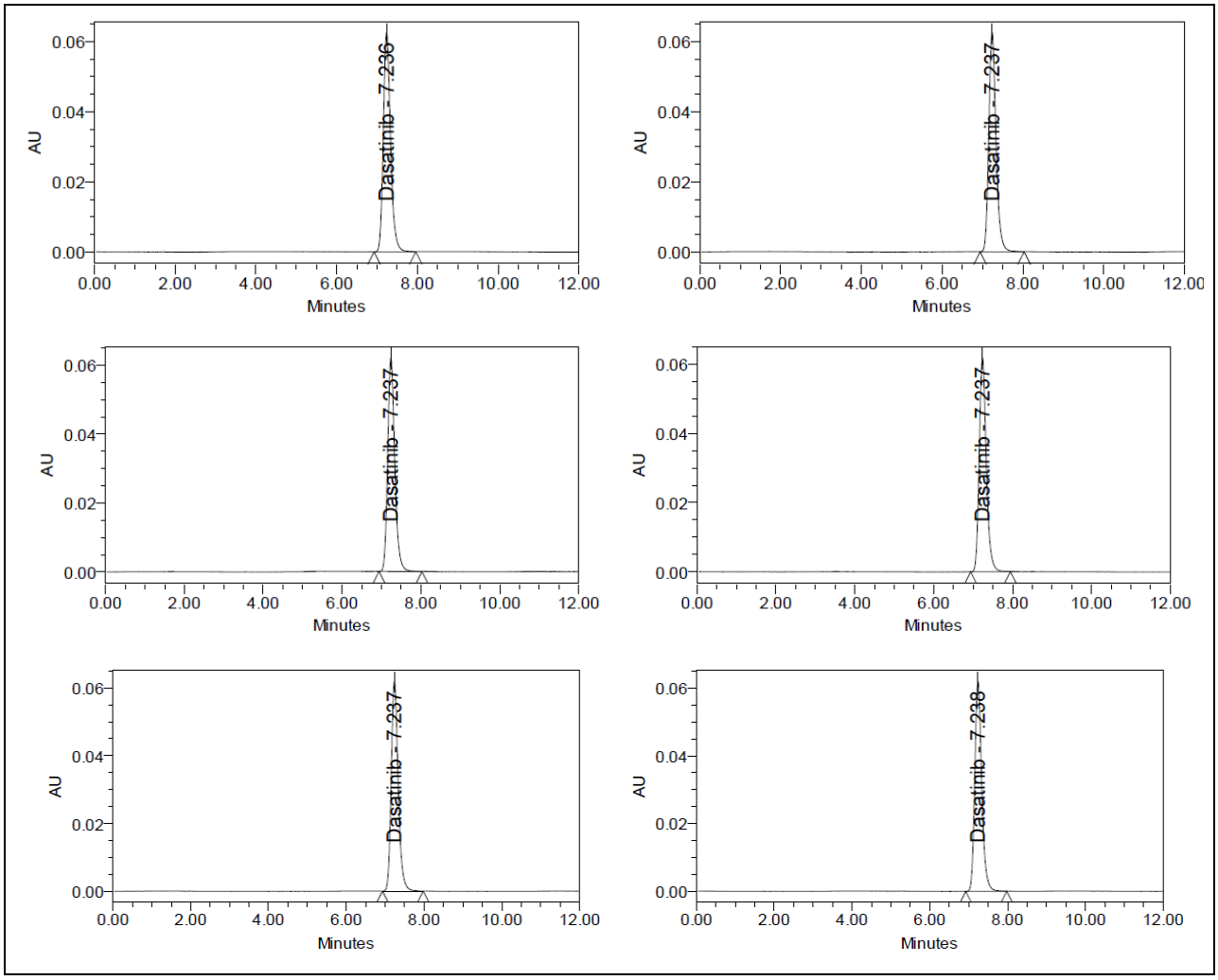

Figure 6: Typical HPLC Chromatograms of Dasatinib tablets for System Suitability.

Accuracy

Accuracy for the assay of Dasatinib tablets was determined by applying the study of recovery Dasatinib tablets, 50mg was conducted on placebo powder which equivalent to about 20%, 50%, 100%, 150% and 300% of the target assay concentration of Dasatinib in Dasatinib tablets,50 mg. Sample solutions were prepared in triplicate for each level and analyzed as per test method. The % recovery was given in **Table** 6. The mean recoveries of Dasatinib from spiked were found to be in the range of 98.5 - 99.8 %.

Ruggedness

A study to establish the stability of Dasatinib in standard and test solutions were conducted on bench top and refrigerator at Initial, 1 day and 2 day. The assays of Dasatinib in standard and test solutions were estimated against freshly prepared standard each time. From the above study, it

was established that the Standard and sample preparations are stable for a period of 48 hours at room temperature (25°C±2°C) and 48 hours at refrigerator condition (2°C-8°C).

Table 6: Accuracy in the Assay Determination of Dasatinib

Sample No.	Concentration Level	'mcg/ml' Added	'mcg/ml' Recovered	% of Recovery	Average % Recovery
1.	20 %	3.924	3.874	98.73	99.8 %
		3.928	3.940	100.31	
		3.919	3.930	100.28	
2.	50 %	9.610	9.489	98.74	98.8 %
		9.602	9.519	99.14	
		9.596	9.445	98.43	
3.	100 %	19.104	18.998	99.45	99.4 %
		19.238	19.165	99.62	
		19.277	19.137	99.27	
4.	150 %	28.608	28.585	99.92	99.7 %
		28.608	28.554	99.81	
		28.742	28.568	99.39	
5.	300 %	57.600	56.738	98.50	98.5 %
		57.562	56.566	98.27	
		57.658	56.998	98.86	

Table 4: Linearity Graph for Dasatinib Assay

% Level	Con. mcg/mL	Area	Y-Best fit	(Difference)²	Correlation Coefficient	Value
20	3.821	155829	149998	34004905	Regression coefficient	1.000
50	9.552	391288	384775	42418594	Y-Intercept	-6534.3
80	15.284	615931	619593	13413371	Slope	40966.2
90	17.194	698548	697839	502808	Sum of squares	417867989.7
100	19.105	779121	776125	8973859	Minimum Value	3.821
110	21.015	852237	854371	4553288	Maximum Value	57.314
120	22.926	931356	932657	1693364	Y-intercept at 100 %	-0.8
150	28.657	1154891	1167435	157344655		
200	38.209	1551923	1558744	46526852		
300	57.314	2351817	2341404	108436294		

Robustness

A study to establish the effect of variation in mobile phase composition, flow, temperature and pH of Buffer in mobile phase was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated. From the above study the proposed method was found to be robust.

Table 3: Results for Range

% Level	Concentration (mcg/mL)	Peak Area
20	3.821	155829
100	19.105	779121
300	57.314	2351817
Correlation Coefficient (r)		1.000

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Dasatinib and can be reliably adopted for routine quality control analysis of Dasatinib in Bulk and its pharmaceutical formulations.

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