A Stability Indicating Method Development and Validation for the Estimation of Dasatinib in Its Api Form And Tablet Dosage Form

R. Naganjaneyulu[1], Boddem Nagesh[2], Derangula Madhuraju[2], Ganta Chandra Lekha[2], Kavitha[2]*

1- Associate professor, Department of Pharmacognosy, Avanthi Institute of Pharmaceutical Sciences, Hayathnagar, Gunthapally, Hyderabad, Telangana-501512

2-Students, Department of Pharmaceutical Analysis, Avanthi Institute of Pharmaceutical Sciences, Hayathnagar, Gunthapally, Hyderabad, Telangana-501512

ABSTRACT :

An Analytical, accurate, precise, efficient and simple RP-HPLC method has been developed and validated for the determination of Dasatinib in bulk and marketed pharmaceutical dosage forms. The mobile phase used for the chromatographic runs consisted of Acetonitrile and Phosphate buffer (0.01M, pH-3.2)in the ratio of 30:70% v/v. The Chromatographic Separation was achieved on a Symmetry C_{18} ODS (4.6mm×250mm) 5µm particle size column using isocratic mode. Drug peak were well separated and were detected by a UV detector at 246 nm. The developed method was linear at the concentration range 6–14 µg/ml for Dasatinib. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. Dasatinib Limit of detection (LOD) and Limit of Quantification (LOQ) were 0.487µg/ml and 1.477µg/ml respectively.As a result, the suggested method with excellent specificity, accuracy, precision, linearity and robustness as well as economical was useful for the regular quality control analysis of Dasatinib in bulk and pharmaceutical dosage forms.

Key Words: Dasatinib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

I. INTRODUCTION

Dasatinib Anhydrous is an orally bioavailable synthetic small molecule-inhibitor of SRC-family protein-tyrosine kinases. Dasatinib 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 [1]. 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 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 [2]. 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. The IUPAC Name of Dasatinib is N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-yl]amino]-1,3-thiazole-5-carboxamide [3]. The Chemical Structure of Dasatinib is follows

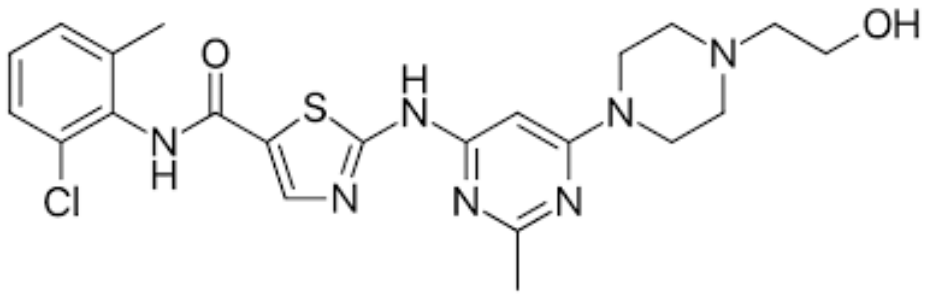

Fig-1: Chemical Structure of Dasatinib

II. EXPERIMENTAL WORK

Materials and Instruments:
The following are the list of instruments/Equipments, chemicals/reagents and standards to perform the HPLC Analysis of the drug Dasatinib [4].

Equipments:
Table-1: List of Equipments

S.No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyser (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Chemicals and Reagents:
Table-2: List of Chemicals used

S.No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

Working Standard: Working Standard of Dasatinib: 10ppm

Method Development:
HPLC Instrumentation & Conditions: The HPLC system employed was **HPLC WATERS** with Empower2 Software with Isocratic with UV-Visible Detector.

Standard Preparation for UV-Spectrophotometer Analysis:
The Standard Stock Solutions – 10 mg of Dasatinib standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration.
Itscanned 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 [5].

Selection of Wavelength:
The detection wave length was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. There sulting solution was scanned in U.V range from200-400nm. The UV spectrum of Dasatinib was obtained and the Dasatinib showed absorbanc smaximaat 246nm. The UV spectra of drug are follows:

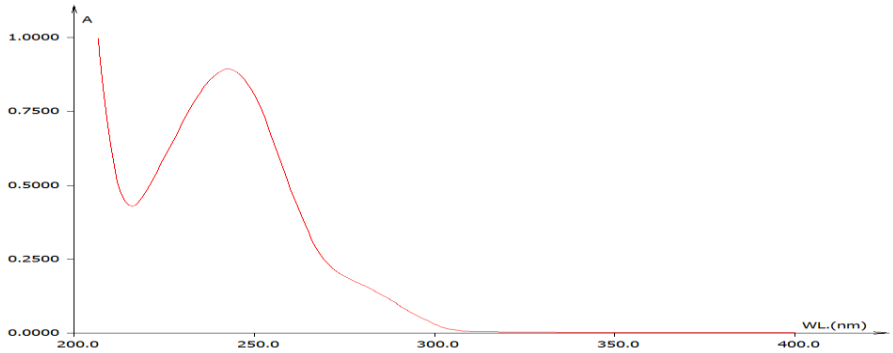

Fig-2: UV Spectrum of Dasatinib (246nm)

Observation: While scanning the Dasatinib solution we observed the maxima at 246nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Selection of Chromatographic Methods:
The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected [6].

Optimization of Column:
The method was performed with various columns like Hypersil C_{18} column, X- bridge column and X-terra (4.6 ×150mm, 5µm particle size), Symmetry C18 ODS (4.6mm×250mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

Mobile Phase Optimization:
Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70respectively [7].

Preparation of Mobile Phase:
Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution:
About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

Diluent Preparation:
Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter [8-9].

Assay
Preparation of the Dasatinibstandard solution:
Preparation of Standard Solution: (Dasatinib)
Accurately weigh and transfer 10 mg of Dasatinib, working standard into a 10ml of clean dry volumetric flasks

add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.
Further pipette 0.1ml of Dasatinib from stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [12,13,18].

Preparation of Sample Solution:
Take average weight of Tablet and crush in a mortar by using pestle and taken weight 10 mg equivalent weight ofDasatinibsample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Procedure:
Further pipette 0.1ml of Dasatinib from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula [10]:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Analytical Method Validation
Validation:
Validation is a process of establishing documented evidence which provide a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics [11, 14-17].

A. System Suitability
System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and theoretical plates [19].

B. Accuracy:
For preparation of 50% Standard stock solution:
Further pipette 0.05ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% Standard stock solution:
Further pipette 0.1ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% Standard stock solution:
Further pipette 0.15 ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Dasatinib and calculate the individual recovery and mean recovery values [20].

Acceptance Criteria:
The %RSD for each level should not be more than 2 .

C. Precision:

Repeatability

Preparation of Dasatinib for Precision:

Further pipette 0.1 ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

D. Ruggedness

To evaluate the intermediate precision of the method, Precision was performed on different days by maintaining same conditions [21].

Procedure:

Day 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

The % RSD for the area of five standard injections results should be not more than 2%.

E. Linearity:

Preparation of Level – I (6µg/ml of Dasatinib):

Further pipette 0.06 ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – II (8µg/ml of Dasatinib):

Further pipette 0.08 ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – III (10µg/ml of Dasatinib):

Further pipette 0.1ml of Dasatinibfrom stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – IV (12µg/ml of Dasatinib):

Further pipette 0.12ml of Dasatinibfrom stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – V (14µg/ml of Dasatinib):

Further pipette 0.14ml of Dasatinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [22].

Acceptance Criteria: Correlation coefficient should be not less than 0.999.

F. Limit of Detection:

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected [23].

G. Limit of Quantitation

The quantification limit is generally determined by the analysis of sample with known concentrations of analyte

and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision [24].

H. Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow Rate:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl 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 i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 70:30, 75:25 instead of 65:35, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded [25].

III. RESULTS AND DISCUSSION

Analytical Method Development:

Optimized Chromatographic Conditions:

Several chromatographic conditions were investigated to obtain an optimum separation with good peak symmetry, satisfactory resolution, and acceptable retention time. Different mobile phase compositions, buffer pH values, flow rates, and detection wavelengths were evaluated during method development. After systematic optimization, the best chromatographic performance was achieved using a mobile phase consisting of Acetonitrile and 0.01 M Phosphate Buffer (pH 3.2) in the ratio of 30:70 (v/v). This composition provided a sharp, symmetrical peak with excellent reproducibility and minimal interference.

Chromatographic separation was carried out on a Symmetry C18 ODS column (4.6 mm × 250 mm, 5 µm particle size). The column exhibited excellent retention characteristics and provided efficient separation of the analyte. A flow rate of 1.0 mL/min was selected as it produced an optimum balance between analysis time and chromatographic efficiency. The UV detector was set at 246 nm, where the analyte exhibited maximum absorbance, ensuring high sensitivity and accurate quantification.

The analysis was performed at ambient column temperature with an injection volume of 20 µL, and the total run time was maintained at 10 minutes. Under these optimized conditions, the analyte produced a well-defined chromatographic peak with acceptable retention time, good theoretical plate count, and minimal peak tailing. The optimized method demonstrated excellent repeatability, sensitivity, and robustness, making it suitable for routine quantitative estimation and subsequent validation studies in accordance with ICH guidelines.

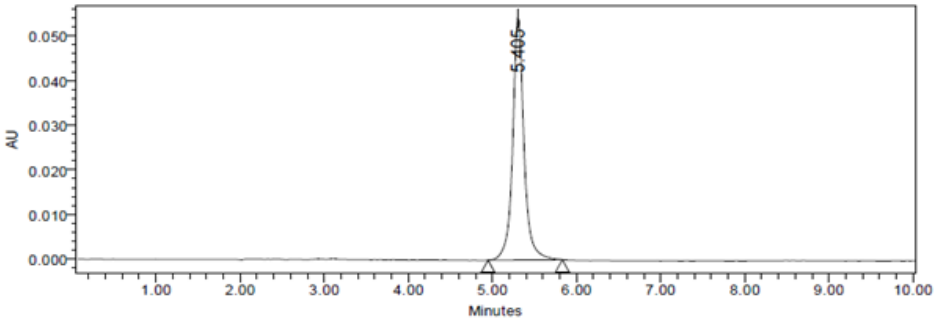

Fig-3: Optimized Chromatographic Condition

Figure-3 shows the optimized chromatogram of the standard solution obtained under the finalized chromatographic conditions. The chromatogram exhibited a sharp, symmetrical, and well-resolved peak without any interference from the mobile phase or other components. The optimized chromatographic conditions ensured consistent retention time, excellent peak shape, and high column efficiency, confirming the suitability of the developed RP-HPLC method for accurate and precise quantitative analysis of the drug in bulk and pharmaceutical dosage forms.

Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution:

About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

Preparationof Standard Solution:
10 mg of Dasatinib working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.
Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).
Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Dasatinib working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Preparation of SampleSolution:
Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Dasatinib equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Validation of Analytical Method:
System Suitability:
Table-3: Observation of System Suitability Parameters

S.No.	Parameter	Dasatinib
1.	Retention Time (min)	5.453
2.	Theoretical Plates	6967
3.	Tailing factor	1.12
4.	Peak Area (AUC)	647856

The system suitability parameters were found to be within the specified limits for the proposed method.

Specificity:
The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Dasatinib in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Observation: The % purity of Dasatinibin present in the marketed pharmaceutical dosage form was found to be 99.85%.

Linearity:
Table-4: Chromatographic Data for Linearity Study of Dasatinib

Concentration µg/ml	Average Peak Area
6	468784
8	615798
10	768759
12	925748
14	1078765

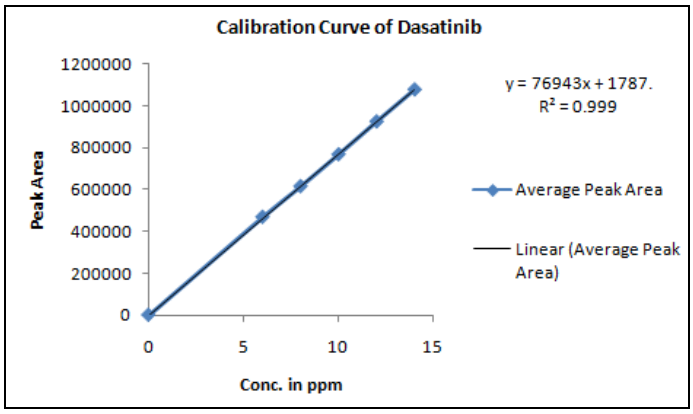

Fig-4: Calibration Curve of Dasatinib

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Dasatinib is a straight line.

Y = mx + c
Slope (m) = 76943
Intercept (c) = 1787
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or Greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 76943. These values meet the validation criteria.

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.

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-5: Results of Repeatability for Dasatinib

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dasatinib	5.419	645784	83685	6825	1.05
2	Dasatinib	5.405	642589	84932	6849	1.09
3	Dasatinib	5.478	643658	85847	6845	1.08
4	Dasatinib	5.466	648759	86295	6839	1.09
5	Dasatinib	5.493	649657	86587	6895	1.07
6	Dasatinib	5.466	647854	87853	6874	1.10
Mean			**646383.5**			
Std. Dev			**2853.319**			
%RSD			**0.441428**			

Intermediate Precision/Ruggedness:

Analyst 1:

Table-6: Results of Intermediate precision for Dasatinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Dasatinib	5.484	636854	84863	6758	1.09
2	Dasatinib	5.493	637489	84759	6726	1.08
3	Dasatinib	5.406	635762	84685	6749	1.09
4	Dasatinib	5.419	636984	84697	6698	1.07
5	Dasatinib	5.446	634856	84258	6728	1.08
6	Dasatinib	5.452	639689	84753	6699	1.08
Mean			**636939**			
Std.Dev.			**1649.149**			
%RSD			**0.258918**			

Analyst2:

Table-7: Results of Intermediate precision Analyst 2 for Dasatinib

S.No.	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Dasatinib	5.491	628985	85698	6985	1.09
2	Dasatinib	5.482	624879	85479	6899	1.07
3	Dasatinib	5.416	625846	85748	6928	1.06
4	Dasatinib	5.482	623568	85647	6874	1.09
5	Dasatinib	5.495	628985	85246	6984	1.07
6	Dasatinib	5.427	628473	85924	6872	1.08
Mean			**626789.3**			
Std.Dev.			**2340.636**			
%RSD			**0.373433**			

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table-8: The accuracy results for Dasatinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	386559	5	5	100.00%	100.13%
100%	768536	10	9.965	99.65%	
150%	1164522	15	15.111	100.74%	

Limit of Detection for Dasatinib:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a samplewhich can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: 0.487µg/ml

Quantitation Limit for Dasatinib:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ=10\times\sigma/S$$

Where,
σ = Standard deviation of the response
S = Slope of the calibration curve

Result: 1.477µg/ml

Robustness:
The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Dasatinib. The method is robust only in less flow condition. The standard of Dasatinib was injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-9: Results for Robustness of Dasatinib

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	648759	5.484	6845	1.08
Less Flow rate of 0.9 mL/min	635248	5.599	6786	1.09
More Flow rate of 1.1 mL/min	659865	4.576	6528	1.05
Less organic phase	625986	7.415	6689	1.03
More organic phase	615869	3.827	6354	1.01

CONCLUSION

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 246nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry C18 ODS (4.6mm×250mm) 5µm particle sizebecause it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile: Phosphate buffer (0.01M, pH-3.2) (30:70 v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 10min because analyze gave peak around 5.405min and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of theDasatinib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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