Research Article

A Novel Stability Indicating Assay Method Development and Validation of Dasatinib Tablets Formulations

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Abstract

A novel isocratic reverse phase liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Dasatinib in bulk drug and its pharmaceutical formulation. Chromatographic separation was achieved on a BDS-C18 column (100 mm × 4.6 mm, 5.0 µ). The mobile phase consisted of 10 mM monobasic phosphate buffer and acetonitrile (67:33v/v) at a flow rate of 1.0 mL min^{-1} and detection was performed at 300 nm using photodiode array (PDA) detector. The drug was subjected to various ICH prescribed stress conditions including hydrolysis (neutral, acid and alkaline), oxidation, photolysis and thermal degradation. The proposed method was validated with respect to specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), stability and robustness as per ICH guideline.

Keywords: Stability Indicating, Validation, Reversed phase, Forced degradation, Dasatinib

The proposed analytical method could effectively separate the drug from its degradation products, employed as stability indicating studies.

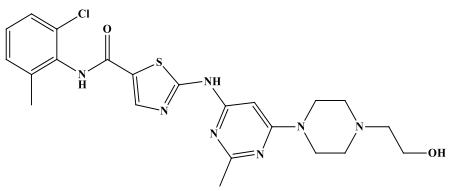

Figure.1. Chemical structure of dasatinib

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Introduction

Dasatinib (*N*-(2-chloro-6-methylphenyl)-2[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4 pyrimidinyl] amino]-5-thiazolecarboxamide monohydrate, BMS-354825) is an oral anti-cancer medication for adults, which belongs to tyrosine kinase inhibitor family [1-3] (Figure. 1). It was architected by chemist Jagabandhu Das from Bristol Myers Squibb Biopharmaceutical Company and sold commercially under the trade name Sprycel[4]. Dasatinib possess a dual SRC/ABL tyrokinase inhibitor, reduces the growth of cancer cells and tumor formation. BMS-354825 is a white powder, melts at 280°–286°C [5-7].

Literature survey revealed a few analytical methods for the stability indicating chromatographic determination of dasatinib by HPLC [8-11], LC-MS [12-16], UPLC [17] in bulk and pharmaceutical formulations. On the basis of these observations, we herewith reported a rapid and sensitive method for stability indicating assay determination.

The aim and objective of this study is to develop and validate an accurate stability indicating RP-HPLC method for the determination of dasatinib in bulk drug and tablet formulations. Furthermore, forced degradation studies were successfully achieved and validated as per the ICH guidelines.

Experimental

Chemicals and Reagents

Pharmaceutical grade of dasatinib supplied as a gift sample by regional bulk drug company, Hyderabad, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. High purity water was prepared by using Milli-Q water purification system.

Instrumentation and Chromatographic conditions

HPLC assay was performed on a Prominence Liquid Chromatograph Waters HPLC PDA 2996 system which consists of a Quaternary solvent manager, a sample manager and water Empower2 software was used to control the equipment and to calculate data and responses from the LC system. The analysis was conducted under using a reverse-phase technique following the conditions: isocratic elution; flow rate of 1.0 mL min^{-1}; mobile phase consisting of 10 mm monobasic phosphate buffer, acetonitrile (67:33; v/v); detector at 300 nm. The mobile phase was prepared, filtered through a 0.45 µm membrane filter (Millipore) and sonicated before use. A Hypersil BDS (100 x4.6 mm i.d., 5 µm particle size) (Thermo Scientific) was used as the analytical column, and the HPLC system was operated at 25 ±1°C.Cintex digital water bath was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India).

Preparation of Stok and Standard solutions

A standard stock solution of Dasatinib (1.0 mg/mL) prepared by dissolving 50 mg of Dasatinib reference standard in 50 mL diluent (acetonitrile: water (50:50, *v/v)*. Required dilutions of stock solution are done to obtain working solution of standard with a concentration of 100µg/mL which is used for the Assay determination (Figure.4).

Preparation of Sample solutions

The tablets of dasatinib are crushed into a fine uniform powder. A quantity of powder equivalent to 20 mg of dasatinib was transferred to a 100 mL volumetric flask, 70 mL diluent was added. The mixture was then sonicated for 20 minute and diluted to volume to give a solution containing 100 µg/mL of dasatinib. The above solution was centrifuged at 4000rpm for 10 minutes in order to eliminate insoluble excipients and filtered through a 0.45 µm pore size Nylon 66 membrane filter and inject in HPLC system as per chromatographic conditions mentioned in section 2.2.(Figure.5).

Analytical Characteristics of the Method

The HPLC method was validated based on specificity, linearity, precision, accuracy, as per ICH guidelines.

Specificity

The specificity experiments were assessed by testing analytical interferences from excipients. The influence of tablet composition was determined by analyzing a placebo solution, submitted to the same extraction procedure and comparing the spectrum to the reference analytical solution. The excipients from original formulation were submitted for extraction and analysis when the drug was completely removed.

Stress degradation were conducted as per the below mentioned ICH guidelines. Dasatinib at a concentration of 1 mg mL^{-1} was used in all degradation studies and samples were stressed from 0-5 days until sufficient degradation was performed. After degradation all solutions were diluted with 70% diluent (v/v) to yield starting concentration of

100µg mL^{-1}, filtered and then chromatographed. The stress conditions used for degradation studies include- acid hydrolysis (refluxed with 1N HCl solution for about 30 min at 80 ºC), alkaline hydrolysis (refluxed with 1N NaOH solution for about 30 min at 80 ºC) and oxidation (treated with 30% H_2O_2 for about 30 min at 80 º C) and finally thermal degradation was also performed.

Linearity

Dasatinib reference solutions were prepared using Diluent in triplicate, at concentrations of 20.0, 50.0, 70.0, 100.0, 120.0 and 150.0µg mL^{-1}. Standard plots were constructed and linearity was evaluated statistically by linear regression analysis using least-squares regression and analysis of variance.

Precision

The repeatability (intra-day and inter-day precision) and intermediate precision (different equipments) of the method were determined as follows: Dasatinib sample solutions were prepared in triplicate on two different days. On each day, the extractive solutions were obtained according to "extraction procedure", using Diluent. The analytical solutions at 100µg mL^{-1} were measured according to established conditions. The results were expressed as the RSD of the analytical measurements. In order to evaluate the intermediate precision, the quantitative solutions were analyzed in equipment using the same parameters.

Accuracy

The accuracy was determined based on the recovery of known amounts of the Dasatinib reference standard added to samples at the levels of 20, 50, 100 and 150% of the sample concentration (100µg mL^{-1}). For this assay, each tablet previously weighed was transferred to 100mL volumetric flasks containing the Diluent and submitted to the extraction procedure for 20 min. The extractive solution at 100µg mL^{-1} was used to prepare the analytical samples in recovery test. This procedure was performed in triplicate. The results were expressed as the percentage of the Dasatinib reference standard recovered from the sample. All solutions were prepared in triplicate.

Results and Discussion

Method development and optimization HPLC Method

In the present work, studies regarding the development and validation of analytical monitoring method for extraction/release of the drug are given. To develop a better way on quantitative method development, the formulation, composition and processing of the drug must be considered. Validated analytical methods for the qualitative or quantitative testing of drug molecules are assumed to be of greater importance when they are employed to generate quality and safety compliance data during the development and post approval of drug products. In our case, a complex formulation of SPRYCEL tablets represents an important challenge in a purpose involving a quantitative analysis by HPLC assay. For accurate analysis, both qualitative and quantitative methods must be developed considering the complexity of the tablet composition, including the use of various excipients such as viscous polymers [30]. Besides the difficulty in accessing the drug due to its location in a polymeric compartment which makes it difficult to assess. It shows that the sample preparation should be given particular attention during analysis. Due to the complexity of excipient matrix in the system, the extraction procedure by ultrasonic bath was reproduced by testing Acetonitrile: Water (50/50 v/v) as Diluent. Thus, the extraction was done to have a final concentration of 100 µg mL^{-1}, and the measurement was performed directly on the final solution. Although Diluent has been confirmed as effective by previous studies during extraction of Dasatinib during the method development, other solvents could be considered, but the results obtained on extraction procedure for HPLC analysis demonstrated that other organic solvents do not promote drug release from matrix. Thus, the main objective of the present work is to develop a stability-indicating assay method for dasatinib bulk drug and its pharmaceutical formulations. A number

of mobile phases and stationary phases were investigated and performed system suitability during the development of assay method. Initially the method development was started with a stationary phase; Hypersil BDS-C_{18} (100 mm × 4.6 mm, 5.0 μ) and mobile phase; 1 mL of ortho-phosphoric acid in 1000ml of water as buffer and acetonitrile in the ratio of 80:20 v/v respectively. Dasatinib peak shape was unsymmetrical. For next trail, the mobile phase ratio was modified slightly in the ratio of 75:25 v/v respectively. In the above trail, the peak shape is broad, but column efficiency was reduced. After few logical attempts, we achieved the best separation; resolution and lower retention time using a Hypersil BDS-C_{18} (100 mm × 4.6 mm, 5.0μ), isocratic reversed phase LC method consist of buffer: acetonitrile in the ratio of 67:33 v/v respectively as mobile phase at flow rate 1.0 mL min^{-1}. The column temperature was maintained at 35 ºC and the detection monitored at 325 nm. The peak shape of dasatinib was found to be symmetrical and retention time is 2.890 min. The system suitability is given in Table 1. The chromatogram of dasatinib standard using the optimized method is shown in Figure 2. The proposed HPLC method was found to be specific for dasatinib and its stressed samples. Dasatinib stressed samples are shown in Fig. 2, 3, 4.

The significant achievements in the proposed method; 1) a sensitive, rapid and isocratic reversed phase HPLC method; 2) lower retention time; 3) best separation and resolution.

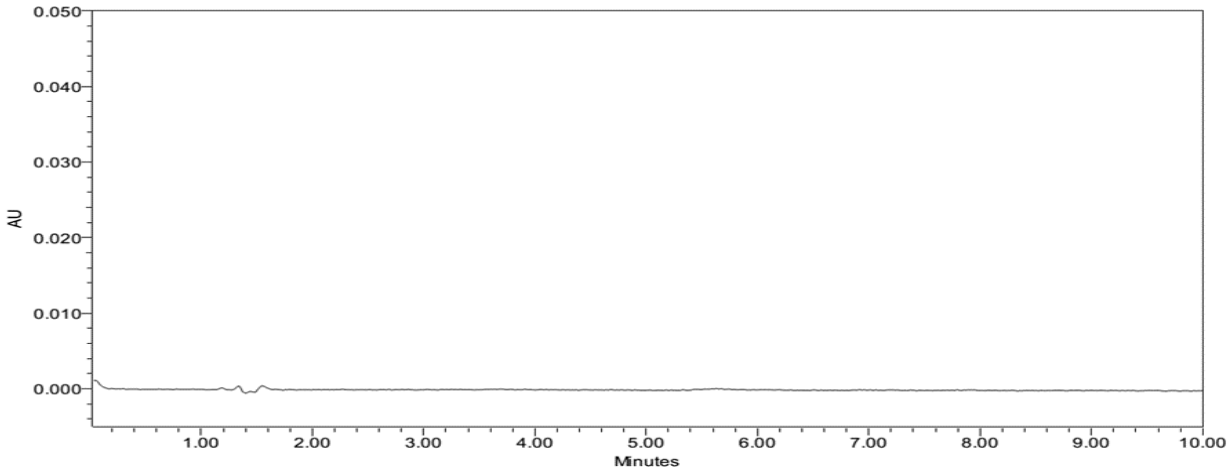

Figure 2 Typical chromatogram of Diluent

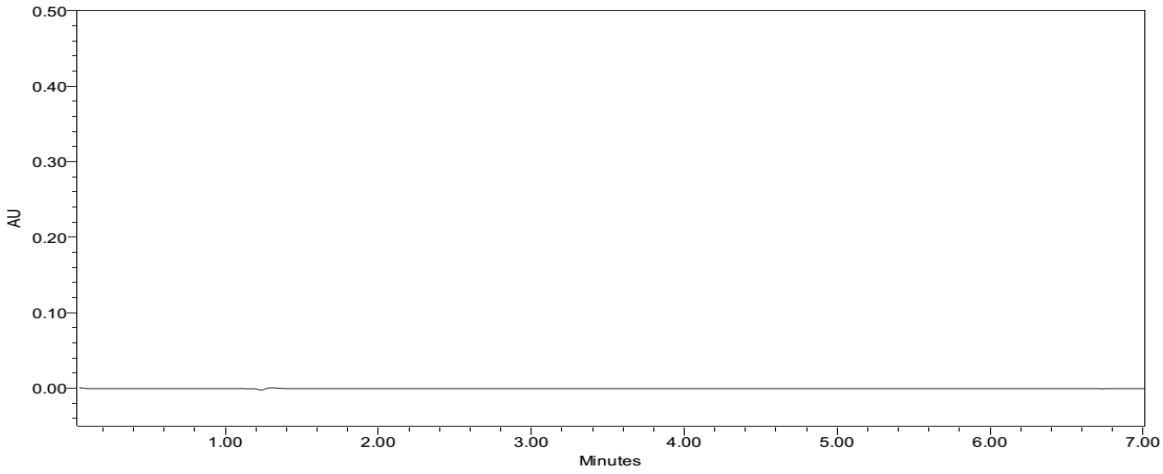

Figure 3 Typical chromatogram of placebo

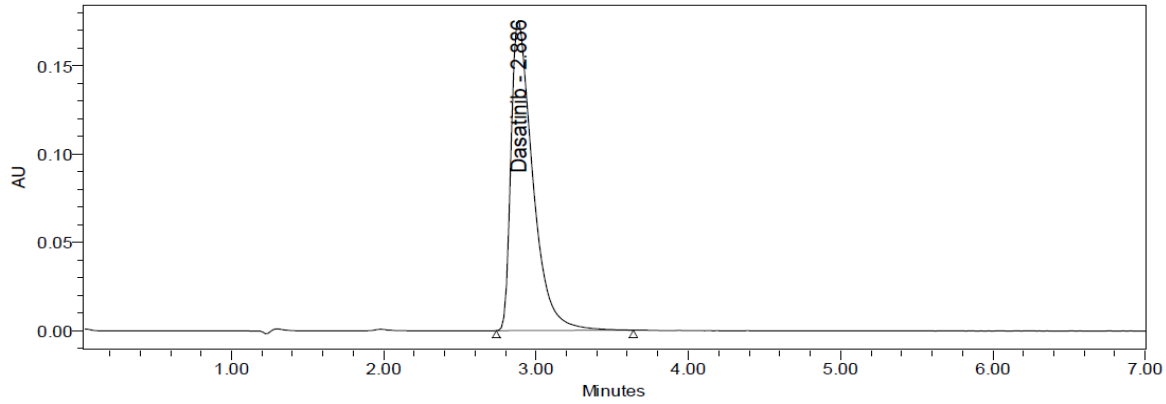

Figure 4 Typical chromatogram of Standard

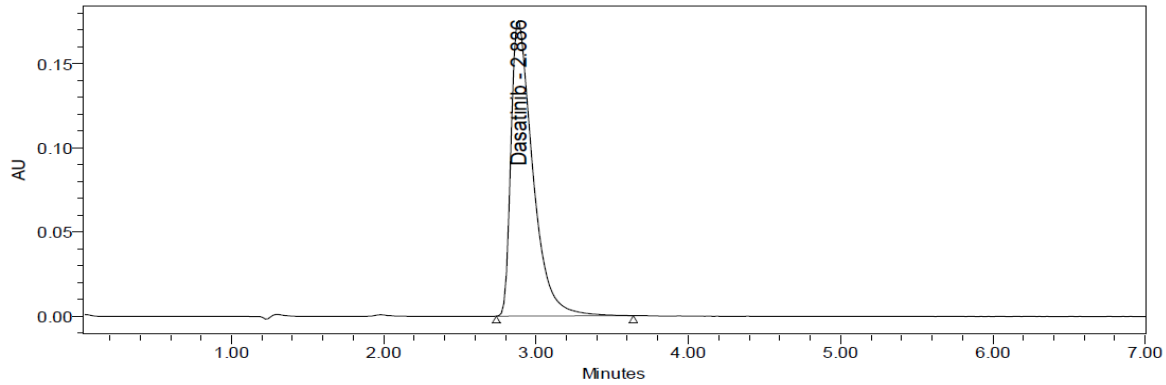

Figure 5 Typical chromatogram of Sample

Method Validation

The above method was validated according to ICH and USP guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.

System Suitability

In order to determine the reproducibility of the proposed methodology, suitability parameters including retention time asymmetry factor, %RSD of retention time and peak areas were investigated. The results are summarized in Table1.

In order to determine the reproducibility of the proposed methodology, suitability parameters including retention time,

Table 1 System Suitability

System suitability parameters	Observed value	Acceptance limit
Retention time	2.94	
USP Tailing	1.79	NMT-2
Theoretical plates	6000	> 3000
% RSD of the Five Standard Injections	0.4	NMT-2.0%

nPrecision

Six replicates (n=6) of sample solutions (100 μg mL^{-1}) were analyzed in the same day to determine method precision. The average % label amount was 99.925% with associated %R.S.D. values of 0.40.

Table 2 System Suitability

Sample No.	% Assay
1	99.42408
2	99.50595
3	100.3031
4	100.0493
5	99.87223
6	100.4003
Mean(X)	99.925
% RSD	0.40

Limit of Detection and Limit of Quantification

The LOD and LOQ of dasatinib were achieved by preparing a standard solution and then analyzing the solutions after carrying out systematic dilutions. LOD and LOQ were determined at a signal-to- noise ratio of 3:0 and 10:0, respectively. The LOD and LOQ concentrations of dasatinib were found to be 0.8 and 2.5μg mL^{-1} for 10μL injection volume and % R.S.D.for the LOQ (n=10) was 1.18%. It is suggested that this method could be used for monitoring dasatinib stability.

Linearity and Range

A series of solutions for testing linearity of dasatinib was performed from 20% level to 150% level of the dasatinib concentration. A plotted linearity graphs of dasatinib standard concentration versus peak area of 20%, 50%, 70%, 100%, 120%, 150% level were plotted and has been found that linearity is in the prescribed range. The correlation coefficients, slopes, and y-intercepts of the calibration plots are reported. The correlation coefficients were >0.999 for all the components. These results shows are an excellent correlation between the peak area and concentration for the drug.

Table 3 Linearity

Sample Name Pipette from stock	Sample ID Volume of flask(ml)	Concentration (ppm)	Peak area
0.2	10	20	360164
0.5	10	50	852137
0.7	10	70	1239656
1.0	10	100	1719446
1.2	10	120	2052274
1.5	10	150	2619313

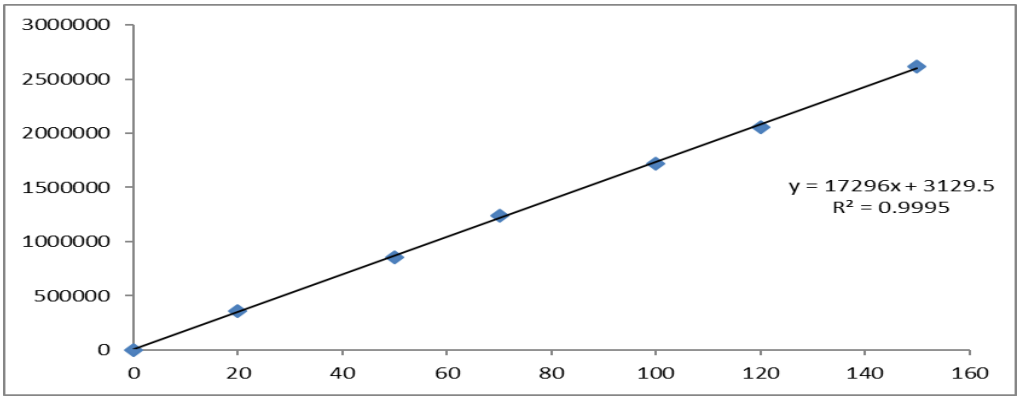

Figure 6 Linearity plot

Accuracy

The accuracy was evaluated by the recovery studies. A series of known amounts of Dasatinib standard was spiked to placebo tablet, mixed and the drug was extracted and diluted to yield five concentration levels: 50,100 and 150 % of the analytical method concentration. Three samples were prepared at each concentration and analyzed. The percentage recovery of added drug was calculated by comparing the peak area of the test samples with that of the standard solutions. The accuracy data is presented in Table 4. The mean recoveries ranged from 99.83-99.9% with % R.S.D. < 1%. Method accuracy was also demonstrated by plotting the amount of Dasatinib found against the amount added over the range of 50-150 % of label amount, the result is found to be satisfactory for intended purpose and is adequate for routine analysis.

Table 4 Accuracy Results of Dasatinib

Spike level	ppm added	ppm found	% Recovery	Mean % recovery	% RSD
50%	50	50.0	100.1	99.9	0.17
	50	49.8	99.8		
	50	49.8	99.8		
100%	100	99.8	99.3	99.83	0.83
	100	99.4	99.4		
	100	100.08	100.8		
150%	150	150.02	100.2	99.9	0.36
	150	150.0	100		
	150	149.99	99.5		

Robustness

To determine the robustness of the method the experimental conditions were deliberately changed and the resolution of Dasatinib is evaluated. To study the effect of flow rate on resolution it was changed to 1.0 and 1.4 mL min^{-1}. The effect of column temperature was studied at 32 ^{0}c and 35 ºC. In all these experiments the mobile phase components were not changed. The effect of the percent organic strength on resolution was studied by varying Acetonitrile by -10

to +10% while other mobile phase components were held constant as stated in Section 2.2. In all the deliberately varied chromatographic conditions the selectivity as well as the performance of the method were unchanged proves thus the robustness of the method.

Stability in solution and in the mobile phase

The stability of Dasatinib standard was assessed by analyzing the solutions (100 μg mL^{-1}) at 0, 2, 4, 6,8,12, 16, 20 and 24 h after preparation. The %R.S.D. value of the assay of Dasatinib was 0.20% (< 1%).The chromatogram showed no peak corresponding to the degradation products and there was no significant change in the peak area response of Dasatinib. The results reveal that standard solution is stable in the mobile phase for at least 24 h. The mobile phase was stable up to 48 h.

Forced Degradation Studies

The drug could be degraded under various stress conditions. Typical chromatograms obtained from stressed samples are illustrated in Fig. (7) and Fig. (12) and the retention time of the degradation products performed from each condition is given in Table 5. The degradation products were well resolved from Dasatinib, confirming the stability indicating power of the method. PDA spectral analysis was employed to verify the homogeneity of the Dasatinib peaks in all degradation sample solutions. The HPLC studied of the stress samples showed the following degradation behavior.

Hydrolytic Degradation Studies

In neutral condition in water at 60°C for 30 min, the drug showed 2.6% degradation and gave a single peak at 2.94 min. under acidic condition in 0.1 N HCl at 60°C for 30.0 min drug showed 7.9% degradation and Two minor degradtion peaks at 1.69 and 2.37 min were formed. In alkaline hydrolysis 2 N NaOH for 30 min, 6.5% of drug was degraded and main analyte peak eluted at 2.94 min. it was suggested that under hydrolysis condition, product show mild degradation. The resulted chromatograms are shown in Figure 7, 8 and 9.

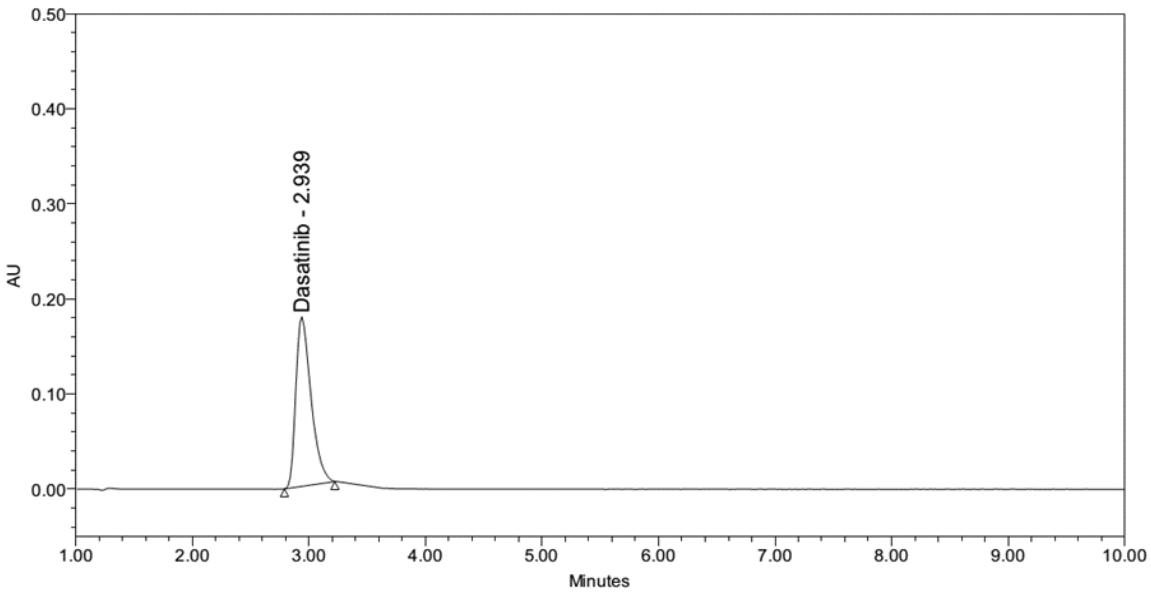

Figure 7 Typical chromatogram of water degradation

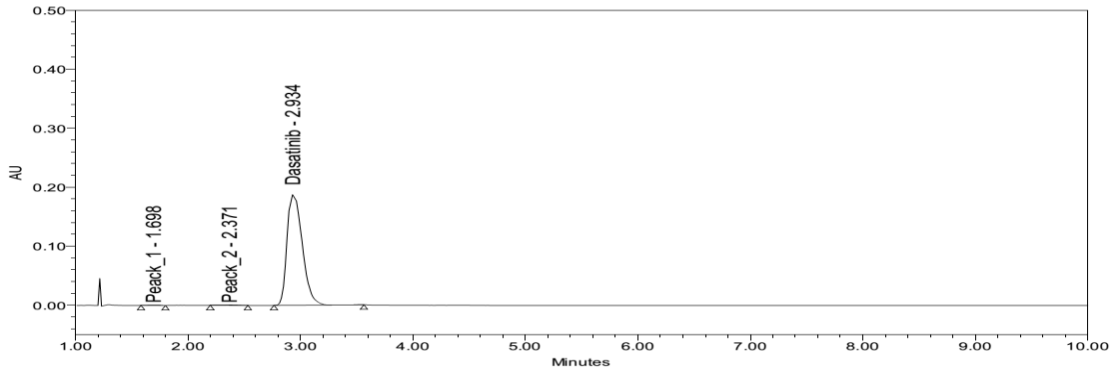

Figure 8 Typical chromatogram of Acidic degradation

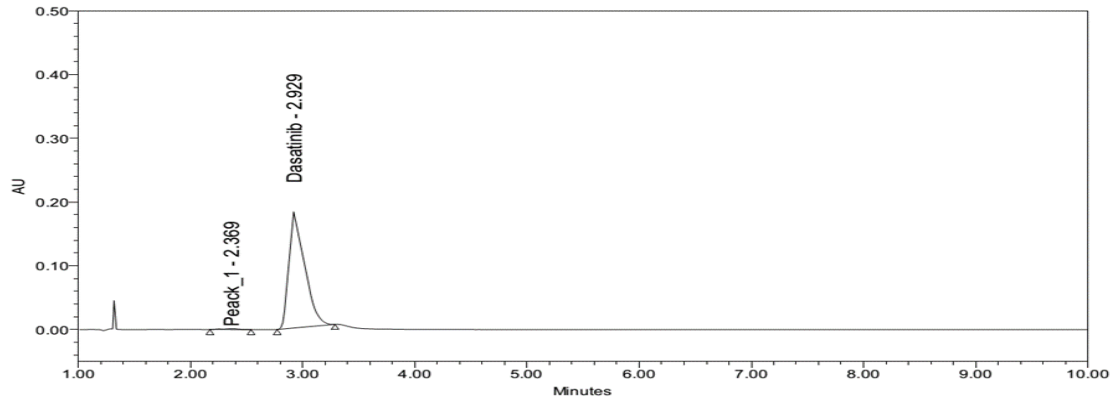

Figure 9 Typical chromatogram of basic degradation

Oxidative Condition

Dasatinib drug was treated with 20% H_2O_2 at room temperature for 60 min and it has not shown any significant sensitivity. The drug was treated with 30% H_2O_2 at 60 ºC for 30 min, the drug shown mild sensitivity. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system. The resulting chromatogram was shown in Figure 10.

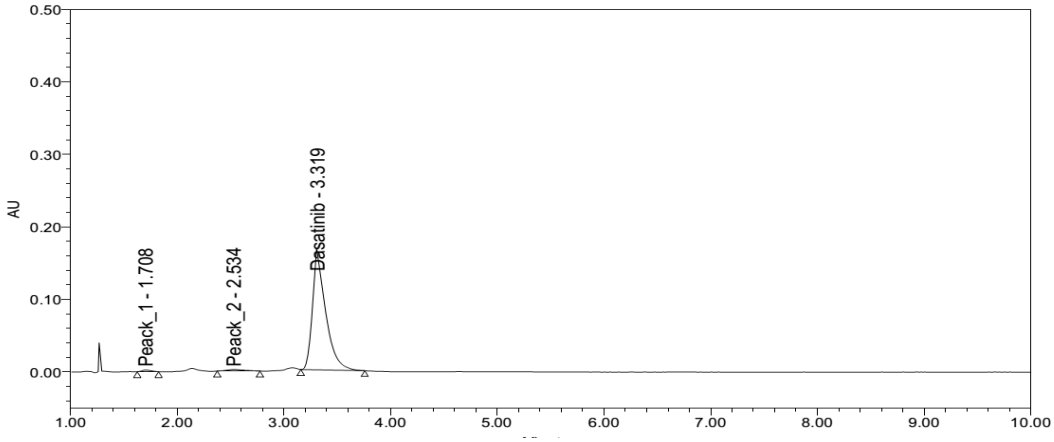

Figure 10 Typical chromatogram of oxidation degradation

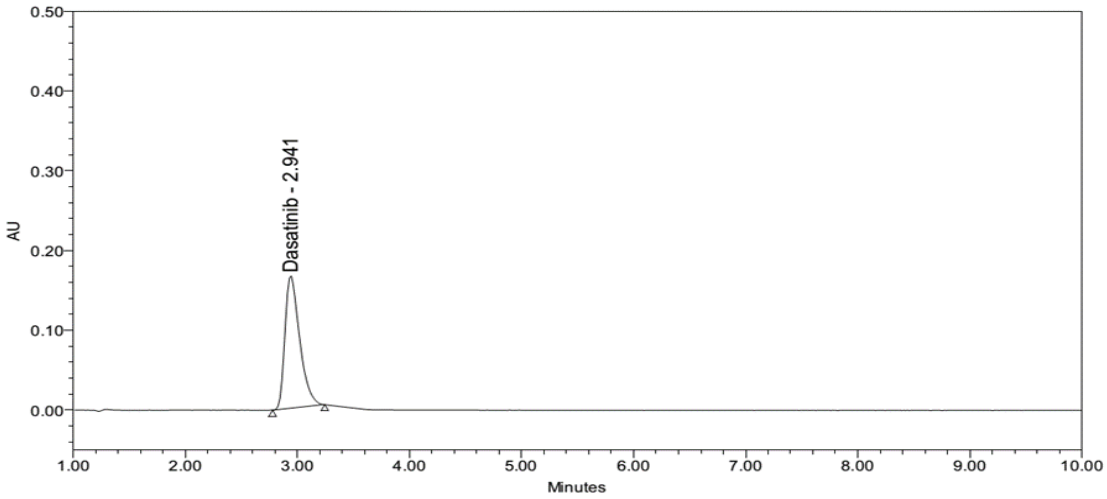

Figure 11 Typical chromatogram of UV light stressed degradation

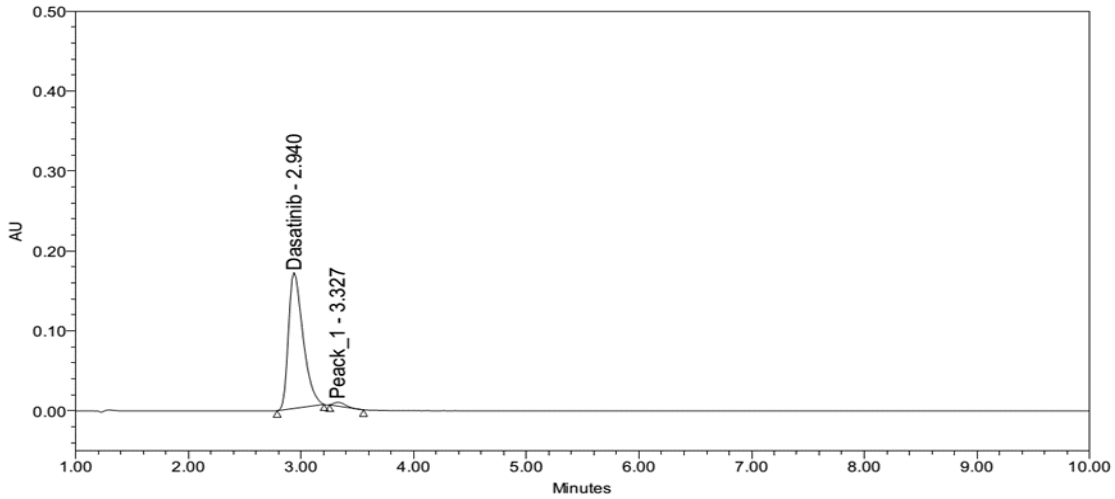

Figure 12 Typical chromatogram of thermal degradation

Photo stability Condition

Dasatinib was stable under acidic, alkaline and thermal degradation conditions. From the above studies, peak purity test results were derived from PDA detector and the drug peak was homogenous and pure in all analyzed stress samples. The mass balance of stressed samples is ~98.9%. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solutions and 10 µl were injected into the system Dasatinib on exposing in UV light (200 wt hours per sq. meter) 1.1% degradation was observed. The resulted chromatogram shown in Figure 11

Degradation in Dry heat

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The resulted chromatogram was shown in Figure 12.

Table 5 Summary of Forced degradation Data

Stress conditions	% Degradation	Drug Product		
		Purity angle	Purity threshold	ASSAY
Control	ND	0.072	0.187	99.98%
Acid hydrolysis	7.9%	0.085	0.273	92.08%
Alkaline hydrolysis	6.5%	0.069	0.254	93.4%
Oxidation	5.6%	0.183	0.275	94.4%
Neutral hydrolysis	2.6%	0.073	0.255	99.3%
Exposed to UV light both at shorter and longer wavelengths for about 200 watt/square meter	1.1%	0.077	0.256	98.9%
Dry heating done at 105^0c for about 2.5 h.	4.8%	0.078	0.258	95.0%

Conclusion

A simple, fast and reliable HPLC method for the quantitative analysis of Dasatinib in tablets was developed and validated. The proposed method presented a good performance and demonstrated to be precise, accurate, sensitive and specific, eliminating the interferences from the polymeric excipients present in matrix tablets. The present method can be used in routine and stability studies during pharmaceutical analysis.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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