International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (3): 232-241
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Validated Reversed Phase High-Performance Liquid Chromatographic Technique for Determination of Dasatanib in Dosage Form: Applications to Stability Studies

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Received: 12 Mar 2020 / Accepted: 10 Apr 2020 / Published online: 01 Jul 2020
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Abstract
An accurate reverse phase High Performance liquid chromatography (RP-HPLC) method has been developed, validated and applied to Stability indicating studies to determine Dasatinib in dosage form. Optimized chromatographic conditions were achieved by using Symmetry, C_{18}, 250mm x 4.6mm.i.d., 5µm Particle sizeas stationary phase and Phosphate Buffer: Acetonitrile = 45:55 (pH-6.2) as eluent at flow rate 1.0 ml/min. UV detection was performed at 305nm. The retention time was found at 4.392 min. The method shows linearity over a range of 25 µg/ml to 150 µg/ml. The correlation coefficient 0.998. The results of system suitability test, linearity, precision, accuracy, robustness, specificity, LOD, LOQ and stabilities found are within the acceptance range. The developed method has been validated statistically as per ICH guidelines. A specific, sensitive, economic stability indicating RP-HPLC method for estimation of Dasatinib has been developed based on ICH Guidelines with bulk and dosage forms. Hence it can be applied for routine analysis of Dasatinib in bulk drug and the Pharmaceutical formulations.

Keywords
Dasatinib, HPLC, Method Development, Retention Time, ICH, Validation, Accuracy, LOD, LOQ, Precision.

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1. INTRODUCTION:
Dasatinib($C_{22}H_{26}ClN_7O2S$) used to treat certain cases of chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL).[1] Specifically it is used to treat cases that are Philadelphia chromosome-positive (Ph+).[1] It is taken by mouth.[1]Common side effects include low white blood cells, low blood platelets, anemia, swelling, rash, and diarrhea.[1] Severe side effects may include bleeding, pulmonary edema, heart failure, and prolonged QT syndrome.[1] Use during pregnancy may result in harm to the baby.[1] It is a tyrosine-kinase inhibitor and works by blocking a number of tyrosine kinases such as Bcr-Abl and the Src kinase family.[1]Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Based on modelling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. [2] In vitro, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib

inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signalling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression. [3]This compound belongs to the class of organic compounds known as aromatic anilides. [4] These are aromatic compounds containing an anilide group in which the carboxamide group is substituted with an aromatic group. [5] They have the general structure RNC (=O) R', where R= benzene, and R = aryl group. The IUPAC Name [6] of Dasatanib is N-(2-chloro-6-methylphenyl)-2-({6-[4-(2-hydroxyethyl) piperazin-1-yl]-2-methylpyrimidin-4-yl} amino)-1, 3-thiazole-5-carboxamide.

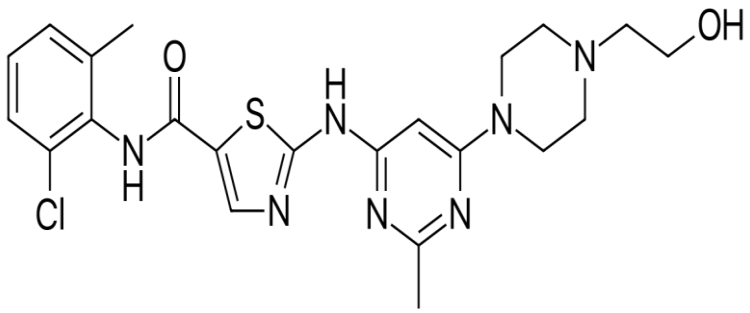


Fig-1: Structure of Dasatanib

A survey of literature [7-16] reveals that very less methods are available for Dasatanib. The present research work describes simple, stability indicating, accurate, specific, robust, rugged and rapid RP-HPLC method developed[17-21] in selected solvent system and validated in accordance with International Conference on Harmonization (ICH) Guidelines Q2 (R1)[22], for the estimation of Dasatanib in bulk drug and in its dosage forms.

2. EXPERIMENTAL:

2.1 Materials and Methods:

Pharmaceutical grade working standard Ruxolitinib were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer(ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Solubility:

Dasatinib was found to be soluble in DMSO, Dimethyl Formamide. Poorly soluble in ethanol and Methanol. Very poorly soluble in water.

2.4 Method Development

2.4.1 Sample & Standard Preparation for the Analysis

25 mg of Dasatinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4.2 Selection of wavelength

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. While scanning the Dasatinib solution we observed the maxima at 305 nm.

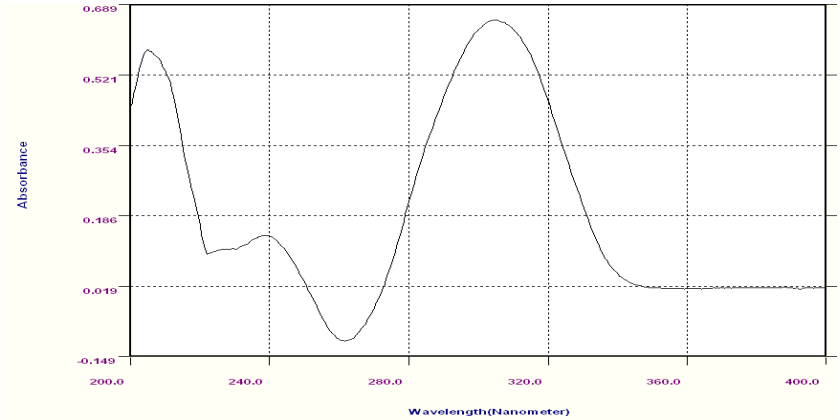


Fig-2: UV Spectrum for Dasatinib

2.4.3 Preparation of 0.05M Phosphate buffer Solution:

About 6.8043 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 6.20 with diluted ortho phosphoric acid

2.4.4 Preparation of Mobile Phase:

The mobile phase used in this analysis consists of a mixture of Phosphate Buffer (pH adjusted to 6.20 with ortho phosphoric acid) and Acetonitrile in a ratio of 45:55.550mL (55%) of Phosphate Buffer (pH adjusted to 6.20 with ortho phosphoric acid) and 450mL of Acetonitrile (45%) of above prepared phosphate buffer were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

2.4.5 Optimized Chromatographic Conditions

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer: Acetonitrile = 45:55 (pH-6.2)
Column	Symmetry, C_{18}, 250mm x 4.6mm .i.d., 5µm Particle size
Flow rate	1.0 ml/ min.
Wavelength	305nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	20µl
Run time	09 min.
Mode of Separation	Isocratic

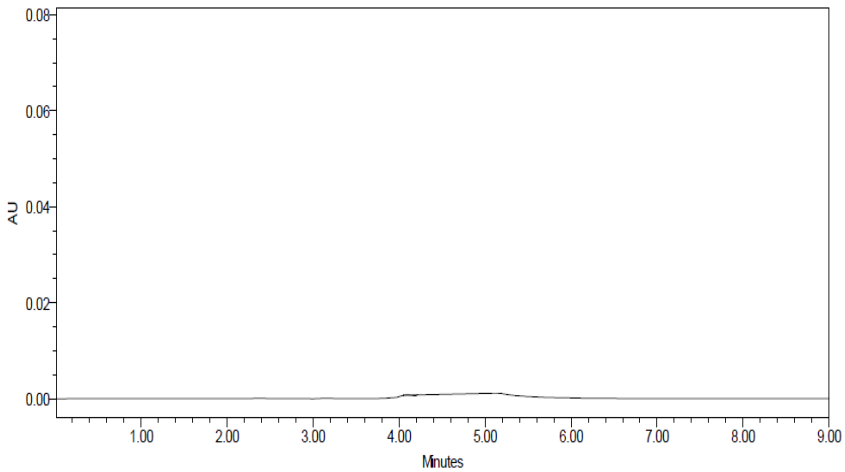
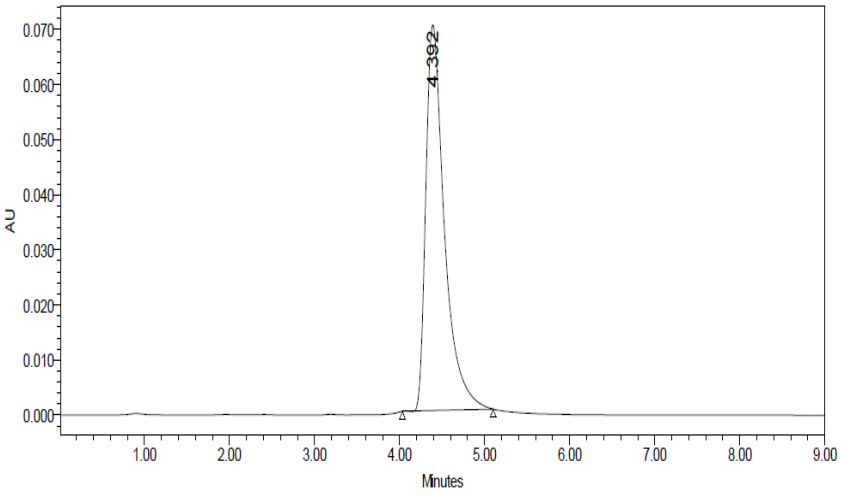


Fig-3: Chromatogram for Blank Preparation



Fig–4: Chromatogram of Dasatinib in Optimized Condition

2.5 Method Validation

2.5.1 Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of pure drug of DASATINIB were taken and added to the pre-analysed formulation of concentration 100µg/ml. From that percentage recovery values were calculated. The results were shown in table-2.

Table-2: Accuracy Readings of Dasatinib

Conc.	Rt	Peak area	Conc. Found	% Recovery
80		5087875	80.075	100.093
80		5078746	79.931	99.913
80		5098749	80.247	100.308
			Avg.	100.1047
			SD	0.197758
			%RSD	0.197551
Conc.	**Rt**	**Peak area**	**Conc. Found**	**% Recovery**
100		6285749	99.010	99.01
100		6324121	99.617	99.617
100		6299879	99.233	99.233
			Avg.	99.28667
			SD	0.307038
			%RSD	0.309244
Conc.	**Rt**	**Peak area**	**Conc. Found**	**% Recovery**
120		7565523	119.240	99.366
120		7658787	120.714	100.595
120		7658749	120.713	100.594
			Avg.	100.185
			SD	0.709275
			%RSD	0.707965

2.5.2. Precision:

2.5.2.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Dasatinib (API). The percent relative standard deviation was calculated for Dasatinib are presented in the Table-3.

Table-3: Repeatability Readings of Dasatinib

HPLC Injection Replicates of Dasatinib	Peak Area
Replicate – 1	6130775
Replicate – 2	6122268
Replicate – 3	6164471
Replicate – 4	6143413
Replicate – 5	6191960
Average	**6150577**
Standard Deviation	**28064.38**
% RSD	**0.456289**

2.5.2.2. Intermediate precision:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Dasatinib revealed that the proposed method is precise.

Table-4: 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=3)	% RSD	Mean (n=3)	% RSD
80	79.98	1.04	80.02	0.23
100	100.95	0.58	100.09	0.47
120	119.82	0.19	119.91	0.19

2.5.3. Linearity & Range:

Calibration standards at five levels were prepared by appropriately mixed and further diluted standard stock solutions in the concentration ranges from 25-150µg/mL for Vilazodone. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the linearity graphs. Chromatograms of each solution were recorded.

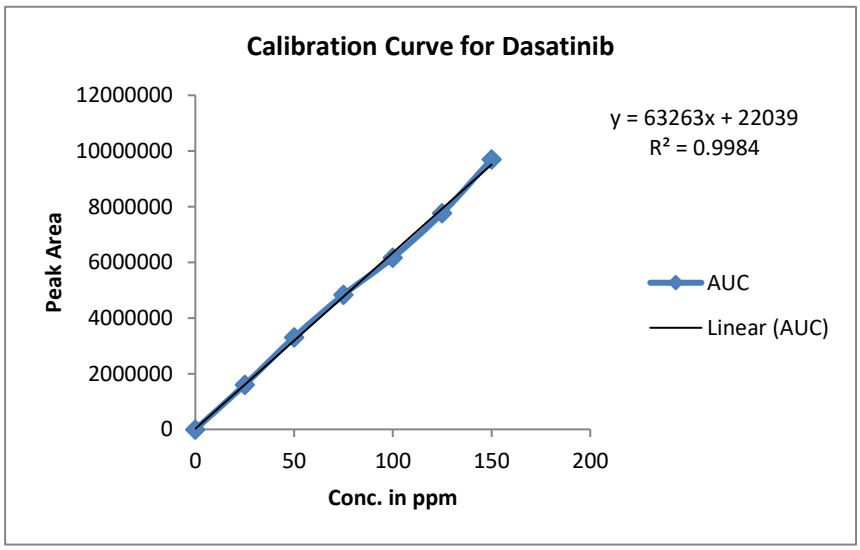


Fig-5: Calibration Curve of Dasatinib (API)

Table-5: Concentration of Dasatinib

CONC.(µg/ml)	AUC
0	0
25	1599571
50	3307873
75	4831264
100	6164471
125	7765523
150	9698441

2.5.4. Method Robustness:

Influence of little changes in optimized chromatographic conditions like changes in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) and Acetonitrile content in mobile phase (±2%) studied to measure the robustness of the method are also in favour of (Table-36, % RSD < 2%) the developed RP-HPLC method for the analysis of Dasatinib (API).

Table-6: Results of method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.15
Flow (0.9 ml/min)	0.84
Temperature (27^{0}C)	0.76
Temperature (23^{0}C)	0.34
Wavelength of Detection (307 nm)	0.27
Wavelength of detection (303 nm)	0.19

2.5.5. LOD & LOQ:

The detection limit (LOD) and quantitation limit (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

2.5.6. System Suitability Parameter

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 analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-7.

Table-7: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.95
2	Tailing Factor	T ≤ 2	Dasatinib=0.99
3	Theoretical plate	N > 2000	Dasatinib=4895

2.5.6. Assay of Dasatinib InPharmaceutical Dosage Form:

Twenty tablets were taken and the I.P. method was followed to calculate the average weight. Above weighed tablets were finally powdered and triturated well. Some quantity of powder which is equivalent to 25 mg of drug was transferred to a clean and dry 25 ml volumetric flask, make and solution was sonicated for fifteen minutes. Then the volume was made up to 25 ml with the same Mobile Phase. Then 10 ml of the prepared above solution was diluted to 100 ml with the help of mobile phase. The resulted solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The final solution prepared was injected in 5 replicates into the HPLC system and the s are record the observations. Two injections of the standard solution were also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.Assay was performed as described in previous chapter. Results obtained are tabulated below:

Table-8: Assay of DASATINIB Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Sprycel Tablets (Actiza Pharmaceutical Private Limited)	50	49.89 (±0.07)	99.76 (±0.68)

2.6 Accelerated stability studies:

The API (Dasatinib) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

Acid degradation: The API was exposed to acidic conditions by using 30 ml of 0.1 N HCl and was refluxed in a water bath at 60^{0}C for 4 hours.

Basic degradation: The API was exposed to acidic conditions by using 30 ml of 0.1 N NaOH and was refluxed in a water bath at 60^{0}C for 4 hours.

Thermal degradation: The drug was mixed with water and refluxed in a water bath at 60^0 c for 6 hours uninterruptedly.

Photolytic degradation: 10 mg of pure drug was taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption

Oxidative degradation: The drug was exposed to oxidative degradation conditions by using 3% H_2O_2& then kept as such in dark for 24 hours.

For all the degradation conditions the final concentration was prepared to100 µg/ml with mobile phase and was injected into the HPLC system.

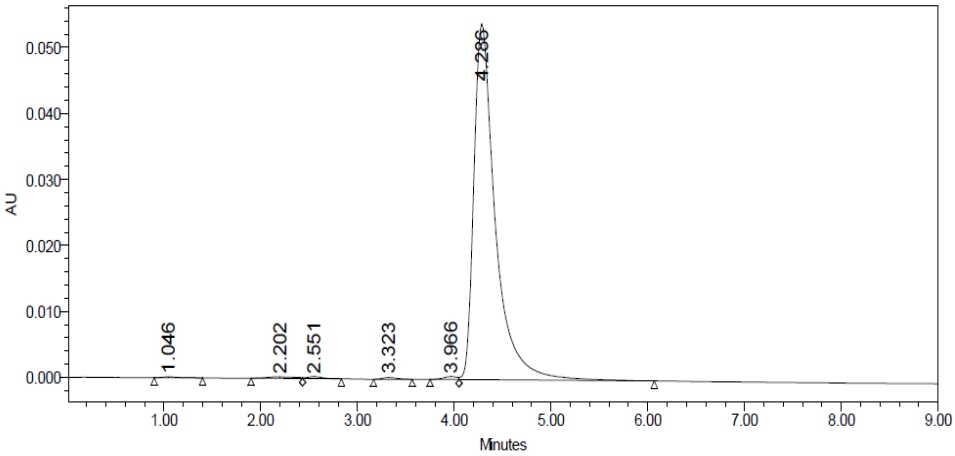


Fig-6: Chromatogram for Acid Degradation

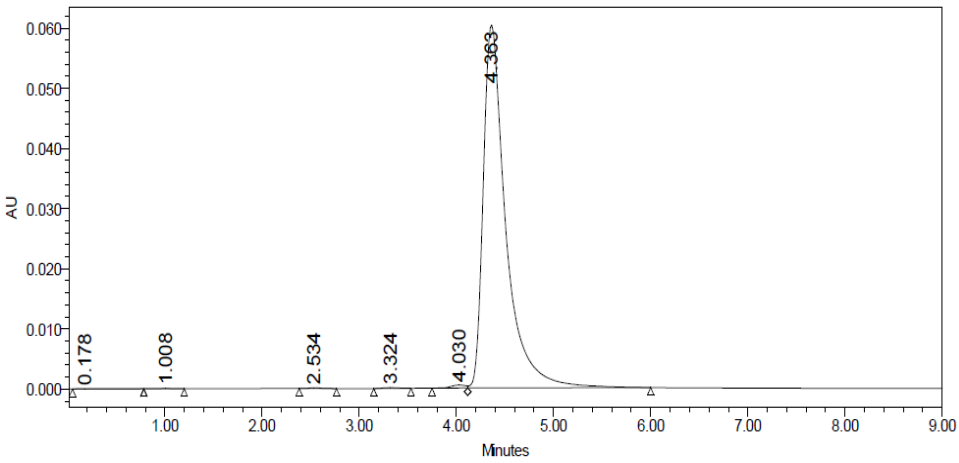


Fig-7: Chromatogram for Basic Degradation

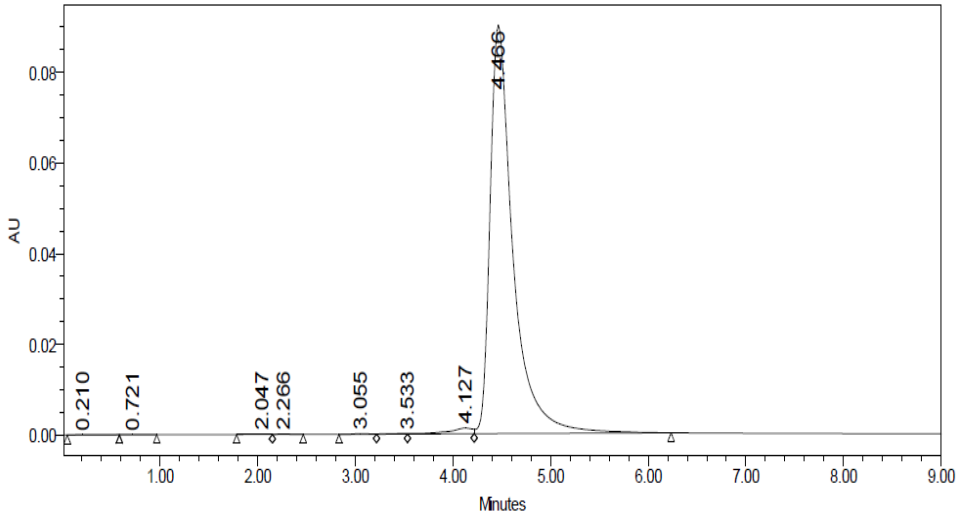


Fig-8: Chromatogram for Thermal Degradation

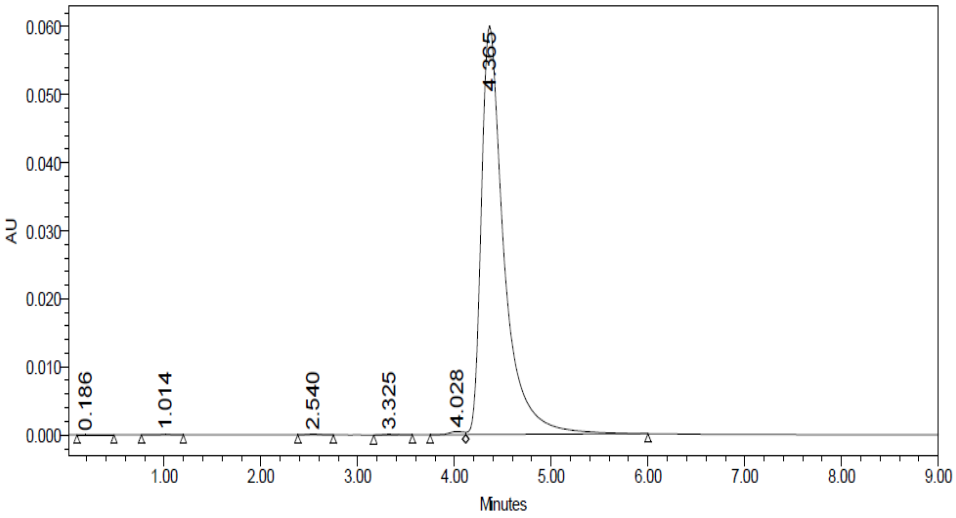


Fig-9: Chromatogram for Photolytic Degradation

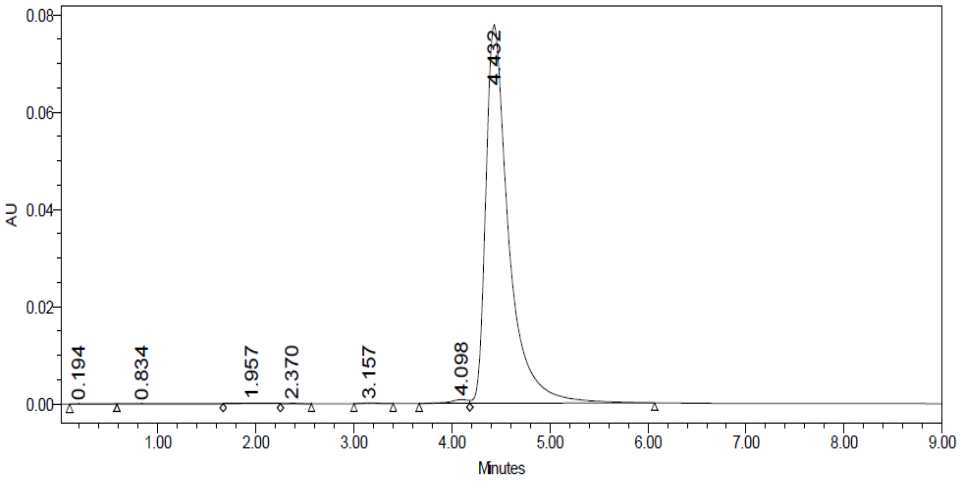


Fig-10: Chromatogram for Oxidation with 3% H_2O_2 Degradation

Table-9: Results of forced degradation studies of Dasatinib API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	4Hrs.	80.94	19.06	100.0
Basic Hydrolysis (0.IM NaOH)	4Hrs.	90.26	9.74	100.0
Thermal Degradation (50 ^{0}C)	6Hrs.	79.54	20.46	100.0
UV (254nm)	24Hrs.	89.68	10.32	100.0
3 % Hydrogen peroxide	24Hrs.	76.29	23.71	100.0

3. RESULTS & DISCUSSION:

To develop a precise, linear, specific stability indicating RP-HPLC method for analysis of Dasatanib, different chromatographic conditions were applied. Isocratic elution was preferred for the current study. In case of RP-HPLC various columns are available, but here Symmetry, C_{18}, 250mm x 4.6mm.i.d., 5µm Particle size column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The retention time was found at 4.39 mins.

The summary of results obtained in method validation were

Linearity & Range: The calibration curve showed good linearity in the range of 0-150 µg/ml, for Dasatinib (API) with correlation coefficient (r^2) of 0.998. A typical calibration curve has the regression equation of y = 63263.x + 22039 for Dasatinib.

Accuracy: The mean recoveries were found to be 100.10, 99.23, 100.18% for Dasatinib. The limit for mean % recovery is 95-105% and as all the values are within the limit, hence it can be said that the proposed method was accurate.

Repeatability: The repeatability study which was conducted on the solution having the concentration of about 100 µg/ml for Dasatinib (n =5) showed %RSD of 0.456%. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.28 µg/ml respectively.

Assay: The assay of Sprycel tablets containing Dasatinib was found to be 99.76%.

Degradation studies: The results of the stress studies indicated the specificity of the method that has been developed. Dasatinib was stable in basic and in photolytic stress conditions as compare to other stress conditions.

4. CONCLUSION:

The data and information concerning drugs, reagents and techniques given in results and discussion reveal that the proposed methods are simple, selective, sensitive (some are superior to the other methods) and accurate with reasonable precision. In addition, selectivity to each selected drug in its formulations was achieved by selecting the appropriate combination of solvent systems, acids or bases in sample solution preparation and exploiting specific functional groups exclusively present in the drug but not in the excipients, additives or other active ingredient present in the formulations, to the extent possible. The proposed method can be used to reported ones and provide a wide choice for the routine determination of the above-mentioned drug depending upon the availability of chemicals and situation arising due to the presence of concomitants.

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