International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Analytical Method Development and Validation of Dasatinib in its Pharmaceutical Dosage Form by UPLC with Forced Degradation Studies

Rao KNV*[1], Srivani P[1], Raja MA[1], Banji D[1], Kumar DS[2]

[1]*Department of Pharmaceutical Analysis & Quality Assurance, Nalanda College of Pharmacy, Charlapally, Nalgonda, Andhra Pradesh, India.*

[2]*School of Pharmacy, Taylors University, Subang Jaya, Malaysia.*

Manuscript No: IJPRS/V2/I4/00230, Received On: 12/12/2013, Accepted On: 15/12/2013

ABSTRACT

A simple, accurate, precise, sensitive, rapid Ultra Performance Liquid chromatography (UPLC) method has been developed and validated for determination of Dasatinib in its pharmaceutical dosage form. Chromatographic separation was achieved on a Waters Acquity BEH C18 column(100 ×2.1mm,1.7), by a mobile phase consisted of Tri Ethyl Amine buffer(pH 6±0.05,maintained with ortho phosphoric acid) and Acetonitrile in 30:70(V/V) ratio with a flow rate of 0.8 ml/min. The detection wavelength was set at 322 nm. Dasatinib was subjected to different stress conditions like acid, alkali, and peroxide, thermal and checked for its specificity, degradation & stability. The method was linear (r = 0.999) at a concentration range of 5-25 µg/ml. The intra and inter day precisions were satisfactory; the relative standard deviations did not exceed 2%. The accuracy of the method was proved; the mean recovery of Dasatinib was 99.04-101.58%**.** The proposed method has high throughput as the analysis involved short run-time (2.5 mins). The method met the ICH/FDA regulatory requirements. The results demonstrated that the method can be applied successfully for routine use in quality control industry laboratories.

KEYWORDS

Dasatinib, UPLC, validation, forced degradation

INTRODUCTION

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reduction in separation time and solvent consumption. Literature reports reveals that UPLC system allows about nine fold decrease in analysis time when compared with conventional HPLC system using 5 µm particle size analytical columns and about threefold decrease in analysis time in comparison to 3 µm particle size analytical column without compromise on overall separation.[1,2] Dasatinib is a tyrosine kinase inhibitor which is used in the treatment of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines over expressing BCR-ABL. It is used for the treatment of imatinib-resistant chronic myeloid leukemia.[3,4] It is available as white to off-white powder , designated chemically as N-(2-chloro-6-methyl phenyl)-2-({6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methyl pyrimidin-4 yl}amino) 1,3-thiazole-5-carboxamide with an empirical formula of $C_{22}H_{26}CLN_7O_2S$ and a molecular weight of 488.01 $g.mol^{-1}$. (Fig.1). Dasatinib is slightly soluble in ethanol, methanol, DMSO, acetone &

***Address for Correspondence:**
Dr. K. N. V. Rao
Professor, Nalanda College of Pharmacy,
Charlapally, Nalgonda, Andhra Pradesh, India.
E-Mail Id: raoknv@yahoo.co.in

Acetonitrile and very poorly soluble in water. It has pKa values of 6.8, 3.1 and 10.8.[5]

Forced degradation studies can be used to provide evidence on how the quality of drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established.[6] Literature survey reveals a few LC-MS methods reported for the determination of Dasatinib and its metabolites in biological fluids[7-10]. A few methods are reported for the determination of Dastinib in pharmaceutical formulations by HPLC, [11-13] HPTLC,[13] UV spectrophotometric,[14] and colorimetric[15] methods ; however UPLC method has not been reported for its estimation till date. Hence an attempt has been made to develop and validate a rapid and sensitive UPLC method along with forced degradation studies for the estimation of Dasatinib as recommended by the International Conference on Harmonization (ICH) guidelines,[16,17] The method was validated by parameters such as linearity, accuracy, precision, LOD,LOQ, robustness.

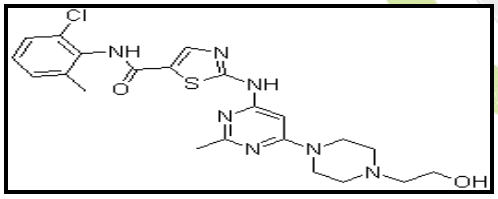

Figure 1: Chemical Structure of Dasatinib

MATERIALS AND METHOD

Apparatus

The analysis of the drug was carried out on a Waters UPLC 2996 separation module equipped with auto sampler& PDA detector.Data was monitored using Empower 2 software. pH meter(Adwa – AD 1020), Weighing machine(Afcoset ER-200A), Pipettes(Borosil),volumetric flasks (Borosil), Ultra sonicator(SV scientific) were used.

Chemicals and Reagents

Dasatinib pure drug was kindly supplied by Natco pharmaceuticals Limited,Hyderabad, India, as gift sample. Commercial dosage forms used is Dasanat 20mg and was purchased from local commercial sources. HPLC grade reagents Acetonitrile, methanol, Triethyl amine, analytical grade ortho phosphoric acid, water-milliQ grade were purchased from Merck India. Pvt. Ltd., Mumbai, India. HCl was obtained from Finar chemical limited, NaOH was obtained from SD fine- chem limited and H_2O_2 was obtained from Alpha Pharma limited.

Preparation of Buffer

Take 1ml Triethylamine (TEA) into a 1000ml beaker, dissolve and dilute to 1000ml with HPLC water and adjust the pH to 6 with ortho phosphoric acid.

Preparation of Mobile Phase

Mix a mixture of above buffer 300mL (30%) and 700 mL of Acetonitrile (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Method Development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer and solvents were evaluated in-order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Finally, following chromatographic conditions were found suitable for the assay.

Optimised Chromatographic Conditions

Instrument: Waters UPLC 2996 separation module equipped with auto sampler& PDA detector

Column: Waters Acquity BEH C18 (2.1 x 100mm, 1.75 µm)

Wavelength: 322nm

Column temperature: Ambient

Flow rate: 0.5 ml/min

Injection volume: 6 µl

Mobile phase: Triethyl amine buffer of pH=6**:** Acetonitrile (30**:**70)

Run time: 2.5 mins

Retention time: 0.828 mins

Technique: Isocratic

Preparation of Mobile Phase

Mix a mixture of above buffer 300mL (30%) and 700 ml of Acetonitrile (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation

Mobile phase is used as diluent

Standard Solution Preparation

Accurately weigh and transfer 10mg of Dasatinib working standard into a 100 ml clean & dry volumetric flask add about 70 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

From the standard stock solution, pipette 1.5 ml of the into a 10ml volumetric flask and dilute up to the mark with diluent to make the working solution of 15µg/ml.

Sample Solution Preparation

Weigh 5 Dasatinib Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Dasatinib into a 100 mL volumetric flask. Add about 70mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluents, thoroughly mix and filter through a 0.45µ pore size injection filter (stock solution).

Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 6 µL of the standard, sample into the chromatographic system and measure the area for the Dasatinib peak and calculate the %Assay by using the formula.

Validation of the Developed Method

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.[16] The proposed method was validated according to the ICH guidelines.

System Suitability Tests

The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can commence. It is used to verify that the reproducibility of the system is adequate for the analysis to be performed. Parameters like %RSD, tailing and theoretical plates are to be taken in to consideration.

Linearity & Range

The response was found linear over a concentration range of 5-25µg/ml for Dasatinib. Accurately measured stock solution of 0.5,1,1.5,2 & 2.5 ml were transferred to a series of 10 mL of volumetric flasks and diluted upto the mark with mobile phase such that the final concentrations are 5,10,15,20,25 µg/mL for Dasatinib. These solutions were injected into chromatographic system, peak area was determined for each concentration of drug solution. Plot a calibration graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area).The correlation coefficients (r), slopes and Y-intercepts of the calibration curve were determined

Accuracy

The accuracy of the method was determined in terms of % recovery. A known quantity of the pure drug was added to the pre analysed sample formulation as 50%, 100% and 150% levels. The recovery studies were carried out 3 times of each level and the percentage recovery was calculated using the formula.

$$\text{Amount found} = \frac{\text{Sample Area}}{\text{Standard Area}} * \frac{\text{Standard concentration}}{\text{Sample Concentration}} * \frac{\%\text{potency of A.P.I}}{100} * \frac{\text{Average weight}}{\text{Label claim}} * 100$$

$$\%\ \text{Recovery} = \frac{\text{Amount found}}{\text{Amount of drug added}} * 100$$

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. Five consecutive injections of 15µg/ml concentration were given on the same day and the values of relative standard deviation (RSD) were calculated to determine intraday precision. The study was also repeated on different day to determine inter day precision.

LOD and LOQ

The limit of detection (LOD) is the lowest detectable concentration of the analyte, while limit of quantitation (LOQ) is the lowest quantifiable concentration. The LOD and LOQ were calculated using the ICH guidelines equation as LOD = 3.3 x σ/S and LOQ = 10 x σ/S, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness of the Method

The optimum chromatographic conditions set for this method have been slightly modified to evaluate the method robustness. The small changes made include the flow rate (±0.05 ml/min) and the organic content in the mobile phase (±10%).

Forced Degradation Studies

Forced degradation studies were performed to evaluate the degradation characteristics of the drug and stability indicating properties of the method**.** The samples of Dasatinib were exposed to acidic, alkaline, thermal and oxidative, and degradation conditions. All the exposed solutions were then analyzed by proposed method. All forced degradation studies were analyzed at 15µg/ml concentration level. Acid degradation was carried out by treating the drug solution with 3ml of 0.1N HCl and alkaline degradation was conducted by treating with 3ml of 0.1N NaOH. After keeping at normal condition for 90mins, the solutions were neutralized and diluted with mobile phase. Solutions for oxidative stress studies were prepared by adding 1 ml of 3% H_2O_2 to the drug solution and keeping aside for 30 mins. The solution was then diluted with the mobile phase. Thermal induced degradation was carried out by adding 3 ml of diluent to the drug solution and was refluxed for 45 mins at 50˚C.The solution was cooled and injected.

RESULTS

System Suitability Tests

The %RSD of five consecutive injections of Dasatinib solutions of 15µg/ml was found to be 0.1%, indicating good injection repeatability. The tailing factor (T) was found to be <2, which reflects peak symmetry; theoretical plate number (N) was found to be 9335 for the column used in the study thus demonstrating acceptable column efficiency. All these results assure the adequacy of the proposed UPLC method for routine analysis of Dasatinib.

Linearity

The correlation coefficient was found to be 0.999 in the given concentration range of 5-25µg/ml. So the method is said to be linear, presented in figure no: 2

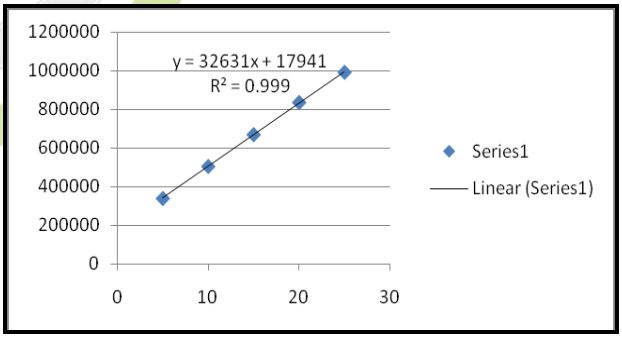

Figure 2: Linearity curve for Dasatinib

Precision

The % RSD values of five consecutive injections of Dasatinib solution of 15µg/ml concentration for both intra-day and inter-day precision was found to be less than 0.3 % and 0.5% respectively which indicates that the proposed method is precise.

Accuracy

The percentage recoveries of Dasatinib ranged from 99.04-101.58%.From the data obtained, it

was observed that the recoveries of standard drugs were found to be accurate and within the specified limits. Results are presented in table no: 1

Table 1: Recovery results of Dasatinib

Con	Area	Amt. Added (mg)	Amt. found (mg)	% recovery	Mean recovery
50	670772.8	5.1	5.12	101.02	100.55%
100	1289436	10.0	9.90	99.04	
150	1997086	15.1	15.3	101.58	

LOD and LOQ

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 0.006 and 0.018 µg/ml, respectively. The results for LOD & LOQ values shows that the method is quite sensitive for Dasatinib

Robustness

Minor deliberate changes in different experimental parameters such as flow rate and wave length did not significantly effect the method indicating that the proposed method is robust. Results are shown in table no: 2.

Forced Degradation Studies

The results of forced degradation studies are given in Table 3.Dasatinib was found more sensitive to oxidative degradation. The assay value was decreased to 74.96 % and degradation peaks were observed in the chromatogram. It was also susceptible to acidic and basic degradations yielding assay values of 83.20 and 88.57 respectively. Comparatively less degradation occurred during thermal degradation yielding an assay value of 93.92%. Chromatograms for the degraded samples for Dasatinib are presented in Figures: 4 to 7.

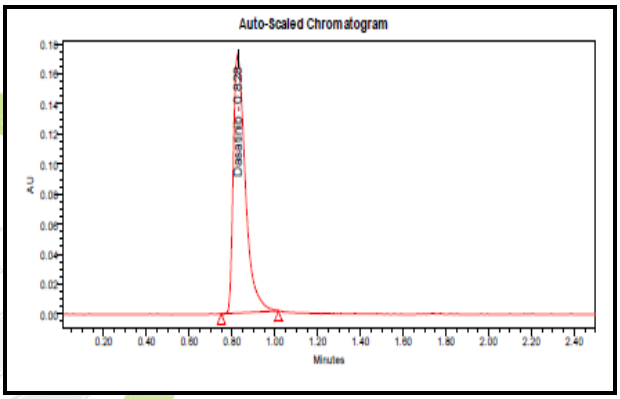

Figure 3: Typical chromatogram showing the standard drug peak of Dasatinib

Table: 2 Robustness results of Dasatinib

Robustness parameters		Retention time	USP plate count	USP tailing
Flow Rate (ml/min)	0.45	1.026	9140	1.93
	0.5	0.829	9319	1.77
	0.55	0.797	9116	1.87
Change in Organic Composition in the Mobile Phase	10% less	1.028	9412	1.79
	Actual*	0.829	9319	1.77
	10% more	0.793	9387	1.82

***** Results for actual Mobile phase composition (30:70 Buffer: Acetonitrile) have been considered

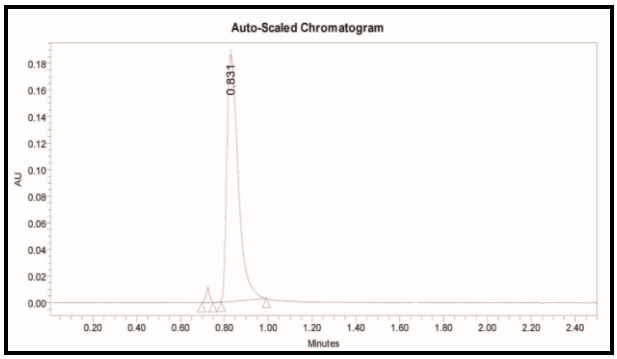

Figure 4: Chromatogram of acid degraded sample of Dasatinib

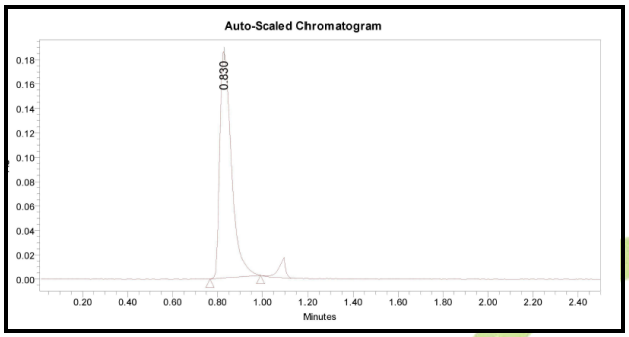

Figure 5: Chromatogram of base degraded sample of Dasatinib

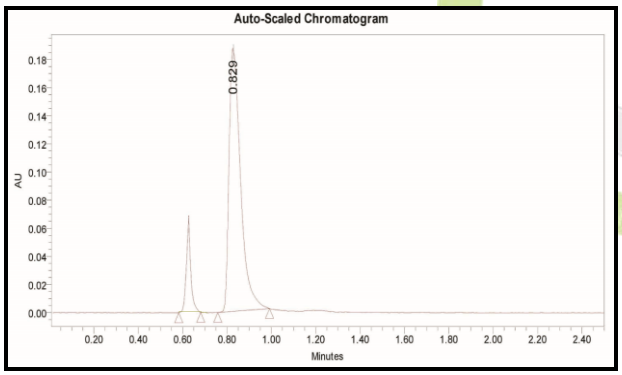

Figure 6: Chromatogram of peroxide degraded sample of Dasatinib

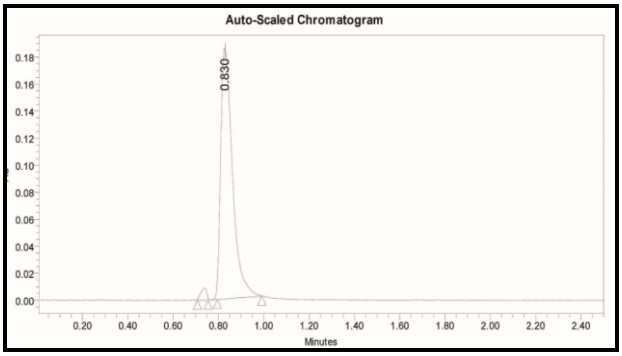

Figure 7: Chromatogram of thermal degraded sample of Dasatinib

Table: 3 Results of forced degradation studies

Stress condition	Sample area	% Assay
Acid degradation	563436	83.50 %
Base degradation	595643	88.27 %
Peroxide degradation	505838	74.96%
Thermal degradation	633799	93.92 %

CONCLUSION

A new method has been established for estimation of Dasatinib by RP-UPLC method. The validation data indicates good precision, accuracy, linearity & reliability of the method. The developed method has advantages like isocratic mode of elution, easy sample preparation, and a short run time of 2.5 mins & allows the analysis of large number of samples in a short period of time. This method can be successfully applied for the routine quality control analysis and stability study of Dasatinib formulations.

ACKNOWLEDGMENT

The authors are very much thankful to Principal and Management, Nalanda College of Pharmacy, Nalgonda for providing all the facilities to do the research work and also I especially thank my guide for the valuable guidance and I am thankful to Natco pharmaceutical Ltd, Hyderabad, A.P, for providing gift sample. Thanks are also extended to my lecturers and friends who helped me throughout my project work.

REFERENCES

1. Wren SA, Tchelitcheff P, "Use of ultra-performance liquid chromatography in pharmaceutical development", Journal of Chromatography A, 2006, 1(6), 119-140.
2. Novakova L, Matysova L, Solich P, "Advantages of application of UPLC in

pharmaceutical analysis", Talanta, 2006, (68), 908-18.

3. Lombardo LJ, Lee FY, Chen P, "Discovery of N- (2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole -5- carboxamide, A dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays", Journal of Medicinal Chemistry, 2004, (47), 6658– 6661.
4. Tokarski JS, Newitt JA, Chang CYJ, "The structure of dasatinib bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib resistant ABL mutants", Cancer Research, 2006, (66), 5790–5797.
5. http://www.drugbank.ca/drugs/
6. Ngwa G, "Forced degradation as an integral part of Hplc stability indicating method development", Drug Delivery Technology, 2010, (5), 10.
7. Pirro E, De Francia S, De Martino F, Fava C, Ulisciani S, Cambrin GR, Racca S, Saglio G, Di Carlo F, "A new HPLC-UV validated method for therapeutic drug monitoring of tyrosine kinase inhibitors in leukemic patients", Journal of Chromatographic Science, 2011, 49(10), 753-759.
8. Lankheet NAG, Hillebrand MJX, Rosing H, Schellens JHM, Beijnen JH, Huitema ADR, "Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry", Biomedical Chromatography, 2012, 27(4), 466-476.
9. Furlong MT, Agrawal S, Hawthorne D, Lago M, Unger S, Krueger L, Stouffer B, "A validated LC-MS/MS assay for the simultaneous determination of the anti-leukemic agent dasatinib and two pharmacologically active metabolites in human plasma: application to a clinical pharmacokinetic study", Journal of Pharmaceutical and Biomedical Analysis, 2012, (58), 130-135.
10. Birch M, Morgan PE, Handley S, A.Ho, Ireland R, Flanagan RJ, "Simple methodology for the therapeutic drug monitoring of the tyrosine kinase inhibitors dasatinib and imatinib", Biomedical Chromatography, 2013, 27(3), 335-342.
11. Kalekar AK, Rao BA, Allamneni Y, Chary PD, Kumar SS, Allamneni N, "Development and Validation of RP-HPLC Method for Estimation of Dasatinib in bulk and its Pharmaceutical formulation", American Journal of PharmTech Research, 2012, 2(4), 863-872.
12. XU Jia-gen, "Determination of Dasatinib and Related Substances in Dasatinib Tablets by HPLC", Chinese Journal of Pharmaceuticals, 2011, (43), 129-131.
13. Mhaske V, Dhaneshwar SR, "Stability Indicating HPTLC and LC Determination of Dasatinib in Pharmaceutical Dosage Form", Journal of Chromatographia, 2001, 66(1), 95-102.
14. Sankar DG, Rajeswari A, Nagesh A, Krishna VM, "Uv-spectrophotometric determination of dasatinib in pharmaceutical dosage forms", Asian Journal of Chemistry, 2009, 21(7), 5777-5779.
15. Vadia N, Rajput S, "Development of colorimetric method for determination of dasatinib in bulk and in tablet formulation", International Journal of Pharmacy and Pharmaceutical Sciences, 2011, 3(2), 188-190.
16. ICH guidelines Q2(R1), Validation of Analytical procedures, Text & methodology, 1995.
17. ICH guidelines Q1A (R2), Stability testing of new drug substances and products, International Conference on Harmonization, 2003.

