INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT AND VALIDATION OF NOVEL HPLC METHOD FOR THE ESTIMATION OF DASATINIB IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate, precise RP-HPLC method was developed and validated for the estimation of Dasatinib in bulk and pharmaceutical dosage forms. A Hypersil BDS C18 column (150 mm x 4.6 mm), 5 µ particle size was used as stationary phase with mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 85:15 V/V. The flow rate was maintained at 1.1 mL/min and effluents were monitored at 300 nm. The retention time was 3.164 min. The linearity of the method was observed in the concentration range of 25-150 µg/mL with correlation coefficient of 0.999. The percentage assay of Dasatinib was 99.93%. The method was validated for its accuracy, precision and system suitability. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the estimation of Dasatinib in pharmaceutical dosage forms.

Keywords: Dasatinib, HPLC, Validation and Formulation.

INTRODUCTION

Dasatinib (Fig. 1) is a kinase inhibitor[1]. Chemically it is N-(2-chloro-6 -methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5thiazole carboxamide, monohydrate. Dasatinib is indicated for the treatment of chronic myelogenous leukemia (CML) and philadelphia chromosome-positive acute lymphoblastic leukemia in chronic phase. Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Based on modeling studies, Dasatinib is predicted to bind to multiple conformations of the ABL kinase[2].

Literature survey revealed that few analytical methods such as HPLC[3-4], LC-MS[5-9] and UPLC[10] methods have been reported for the estimation of Dasatinib. Hence a new sensitive and efficient HPLC method[11] was developed and validated as per ICH guidelines[12] for the estimation of Dasatinib in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Dasatinib using Waters HPLC system on Hypersil BDS C18 column (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with an auto sampler and PDA detector. A 10 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower2 software.

Chemicals and solvents

The working standard of Dasatinib was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation SPRYCEL tablets (Dasatinib 100 mg) were procured from local market. HPLC grade acetonitrile, methanol and water were purchased from E.Merck (India) Ltd, Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

Chromatographic conditions
A mixture of phosphate buffer and acetonitrile in the ratio of 85:15 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Dasatinib. The solvent mixture was filtered through 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.1 mL/min. Injection volume was 10 µL and the column was maintained at a temperature of 30^0C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 300 nm. The run time was set at 6 min.

Preparation of phosphate buffer pH 3.5
2.72 grams of potassium dihydrogen phosphate was weighed and transferred into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water. pH was adjusted to 3.5 with orthophosporic acid.

Preparation of mobile phase and diluent
850 mL of the phosphate buffer was mixed with 150 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The solvent methanol was used as diluent.

Preparation of standard solution
10 mg of Dasatinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 0.25 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentrations.

Preparation of sample solution
Twenty commercial tablets were weighed and powdered. A quantity of the powder equivalent to 10 mg of Dasatinib was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 0.25 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentrations of Dasatinib in pharmaceutical dosage form.

Linearity
Several aliquots of standard solution of Dasatinib was taken in different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Dasatinib were in the range of 25 to 150 µg/mL. Evaluation of the drug was performed with UV detector at 300 nm, peak area was recorded for all the peaks. The correlation coefficient value of Dasatinib was 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Limit of detection and limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD for Dasatinib was found to be 0.23 µg/mL. The LOQ for Dasatinib was found to be 0.72 µg/mL.

System suitability
System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy
The accuracy of the method was assessed by recovery study of Dasatinib in the dosage form at three concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The content of Dasatinib per tablet was calculated. The mean recovery of Dasatinib was in the range of 99.56% that shows there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate.

Precision
The precision was determined for Dasatinib in terms of intra-day and inter-day precision. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and %RSD for Dasatinib was 0.70% (limit %RSD < 2.0%). In addition, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the %RSD for Dasatinib was 0.45% (limit %RSD < 2.0%).

Ruggedness and robustness
The ruggedness of the method was determined by carrying out the experiment on

different instruments by different operators using different columns of similar types. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method so developed is rugged and robust.

Assay

10 µL of each standard and sample solution were injected and from the peak area of Dasatinib, amount of each drug in samples were computed. The result of assay undertaken yielded 99.93% of label claim of Dasatinib.

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop an accurate and precise method in tablet dosage form using Hypersil BDS C18 column (150 x 4.6 mm, 5 µ) in isocratic mode with mobile phase composition of phosphate buffer: acetonitrile (85:15 V/V) and pH adjusted to 3.5 with orthophosphoric acid. The use of phosphate buffer and acetonitrile in the ratio of 85:15 V/V resulted in peak with good shape and resolution. The flow rate was 1.1 mL/min and the drug component was measured with UV detector at 300 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 25 to 150 µg/mL for Dasatinib with correlation coefficient of 0.999. The linearity results were shown in Table 2 and the linearity curve was shown in Fig. 2. The % recovery was found to be 99.56% for Dasatinib, which indicate the method is accurate. The results of recovery studies were shown in Table 3. The %RSD for intra-day precision and inter-day precision for Dasatinib were found to be 0.70 and 0.45, which indicate the method is precise. The results of precision studies were shown in Table 4.

The retention time of Dasatinib was 3.164 min. The number of theoretical plates was 4708 and tailing factor was 1.23 for Dasatinib, which indicates efficient performance of the column. The limit of detection and limit of quantification for Dasatinib were found to be 0.23 µg/mL and 0.72 µg/mL, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 5. Validated method was applied for the determination of Dasatinib in commercial formulations. The %assay was found to be 99.93% for Dasatinib and the assay results were shown in Table 6.

Typical chromatogram of drug Dasatinib was shown in Fig. 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

A simple, accurate, precise, selective and sensitive RP-HPLC method for Dasatinib was developed and validated. This method will be useful for the easy and quick estimation of Dasatinib with almost no interferences in bulk and pharmaceutical dosage forms.

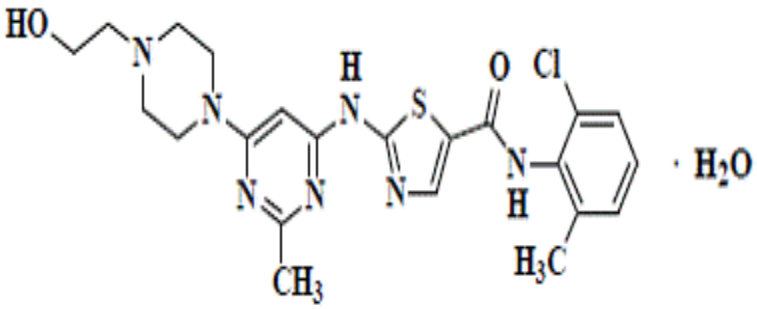

Fig. 1: Chemical structure of Dasatinib

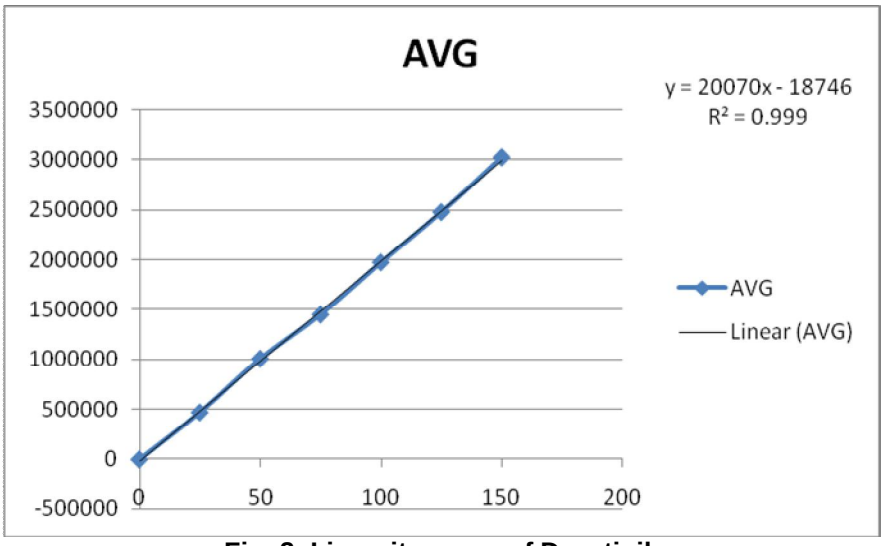

Fig. 2: Linearity curve of Dasatinib

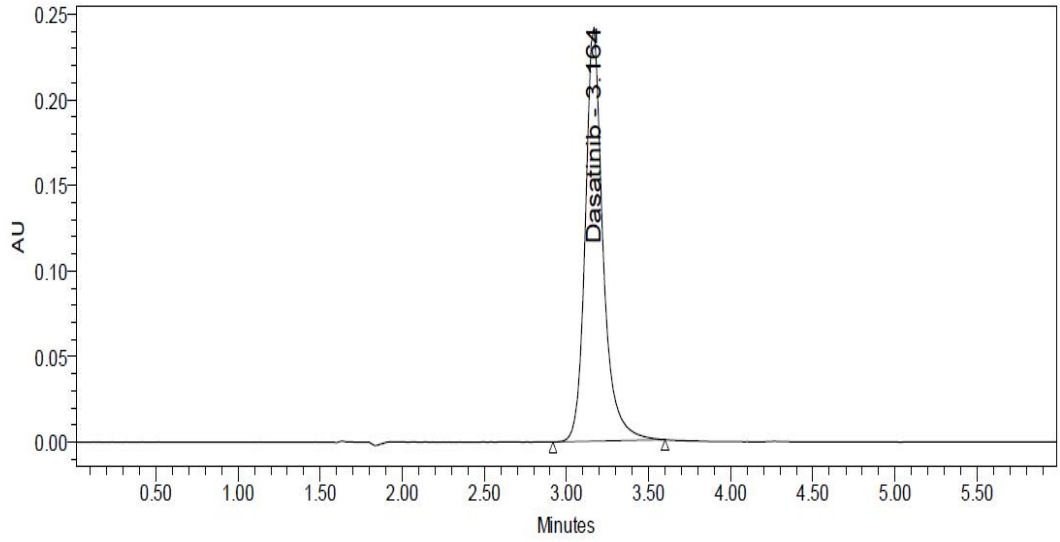

Fig. 3: Typical chromatogram of Dasatinib

Table 1: Optimized chromatographic conditions of Dasatinib

Parameter	Condition
Mobile phase	Phosphate buffer:acetonitrile (85:15, V/V)
pH	3.5
Diluent	Methanol
Column	Hypersil BDS C18 column (150 x4.6 mm, 5µ)
Column temperature	30^0C
Wave length	300 nm
Injection volume	10 µL
Flow rate	1.1 mL/min
Run time	6 min
Retention time	3.164 min

Table 2: Linearity results of Dasatinib

Concentration (µg/mL)	Area
25	470634
50	1008342
75	1451642
100	1974055
125	2478230
150	3022757

Table 3: Recovery results of Dasatinib

Level	Concentration added (µg/mL)	Concentration found (µg/mL)	% Recovery	Mean recovery
50%	50	49.66	99.33%	99.56%
100%	100	99.55	99.55%	
150%	150	149.74	99.82%	

Table 4: Precision studies of Dasatinib

Concentration (µg/mL)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
1000	0.70	0.45

Table 5: Summary of system suitability and validation parameters of Dasatinib

Parameter	Results
Linearity range (µg/mL)	25-150
Correlation coefficient	0.999
Theoretical plates (N)	4708
Tailing factor	1.23
LOD (µg/mL)	0.23
LOQ (µg/mL)	0.72

Table 6: Assay results of Dasatinib

Formulation	Label claim	Amount found	%Assay
SPRYCEL	100 mg	99.85 mg	99.93%

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