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Development and Validation of New RP-HPLC Method for the Estimation of Dasatinib in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, precise, accurate RP-HPLC method was developed and validated for the estimation of Dasatinib in pharmaceutical dosage forms. An Cosmicsil 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: a mixture of acetonitrile and methanol in the ratio of 50:50, v/v. The flow rate was maintained at 1 mL/min and effluents were monitored at 315 nm. The retention time was 6.4675 min. The linearity of the method was observed in the concentration range of 20-60 µg/mL with correlation coefficient of 0.999. The percentage assay of Dasatinib was 100.10%. 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.

Key-words: Dasatinib, Cosmicsil BDS C18 column, RP-HPLC, Validation.

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INTRODUCTION:

Dasatinib (Fig. 1) is chemically N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-yl]amino]-1,3-thiazole-5-carboxamide (1). Dasatinib is an oral dual BCR/ABL and SRC family tyrosine kinase inhibitor approved for use in patients with chronic myelogenous leukemia (2).

Literature survey revealed that few analytical methods such as LC-MS[3-8], UPLC[9], HPLC[10-16] methods have been reported for the estimation of Dasatinib. A new HPLC method was developed and validated as per ICH guidelines[17] for the estimation of Dasatinib in pharmaceutical formulations.

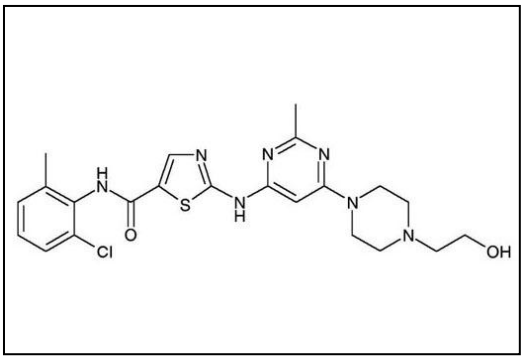

Fig. 1: Chemical structure of Dasatinib

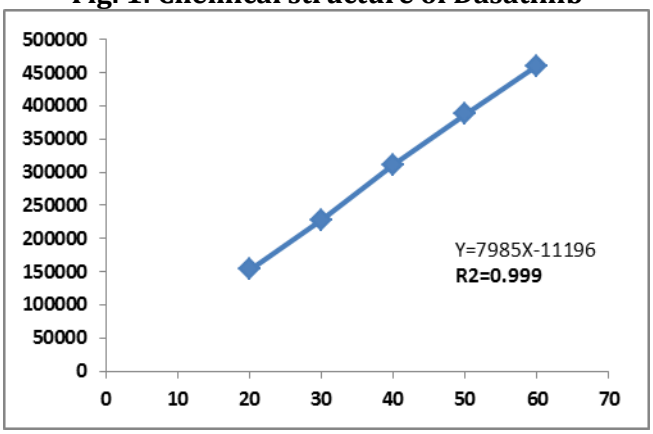

Fig. 2: Linearity curve of Dasatinib

MATERIALS AND METHODS

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Dasatinib using Waters HPLC system on Cosmicsil BDS C18 column (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with an auto sampler and DAD or UV detector. A 20 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 Pharma Train, Hyderabad, India. The market formulation SPRYCEL® tablets were procured from local market. HPLC grade water and methanol were purchased from E. Merck (India) Ltd, Mumbai, India. Triethyl amine and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

Chromatographic conditions

A mixture of phosphate buffer and solvent mixture of acetonitrile and methanol in the ratio of 50:50, v/v was found to be the most suitable mobile phase for ideal chromatographic separation of Dasatinib. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a

flow rate of 1.0 mL/min. Injection volume was 10 µL and the column was maintained at ambient temperature. 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 315 nm. The run time was set at 10 min.

Preparation of phosphate buffer
Added 4.0ml of triethylamine to 100ml water and adjusted the p^H to 6.5±0.05 with dilute orthophosphoric acid.

Preparation of solvent mixture and mobile phase
Prepared a mixture of methanol and acetonitrile in the ratio of 50:50 v/v respectively. Prepared, filtered and degassed mixture of buffer and solvent in the ratio of 50:50 v/v respectively.

Preparation of standard solution
Accurately weighed and transferred the drug equivalent to 50 mg of Dasatinib (working standard) into 50ml volumetric flask. Added about 30ml of solvent mixture and sonicated to dissolve. Cooled the solution to room temperature and diluted to volume with solvent mixture. Transferred 1.0ml of the above solution into a 10ml volumetric flask and diluted to volume with mobile phase.

Preparation of sample solution
10 tablets of marketed formulation was taken, weighed and finely powdered. Accurately weighed and transferred the drug powder equivalent to 50 mg of Dasatinib (working standard) into 50ml volumetric flask. Added about 30ml of solvent mixture, placed on orbital shaker for 15min and sonicated for 30min with occasional shakings. Cooled the solution to room temperature and diluted to volume with solvent mixture. Centrifuged the solution at 3000 rpm for 15min. Transferred 1ml of the above solution into a 10ml volumetric flask, dilutedto volume with mobile phase.

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 20 to 60 µg/mL. Evaluation of the drug was performed with UV detector at 315 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 0.0405µg/mL. The LOQ for Dasatinib was found to be 0.1229 µg/mL.

System suitability
System suitability parameters like retention time, resolution, 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 pre analyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The content Dasatinib per tablet was calculated. The mean recovery of was in the range Dasatinib of 100.16% 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 calculated and was found to be 0.80% (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 calculated and was found to be 0.78% (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 100.1% of label claim of Dasatinib.

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop an accurate method in tablet dosage form using Cosmicsil BDS C18 column (150 x 4.6 mm, 5 µ) in isocratic mode with mobile phase composition of triethylamine buffer and mixture of acetonitrile: methanol (50:50, v/v) in the ratio of 50:50, v/v and pH adjusted to 6.5 with orthophosphoric acid. The use of this mobile phase resulted in peak with good shape and resolution. The flow rate was 1.0 mL/min and the drug component was measured with UV detector at 315 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 20 to 60 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 100.1% 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 0.80 and 0.74, which indicate the method is precise. The results of precision studies were shown in Table 4.

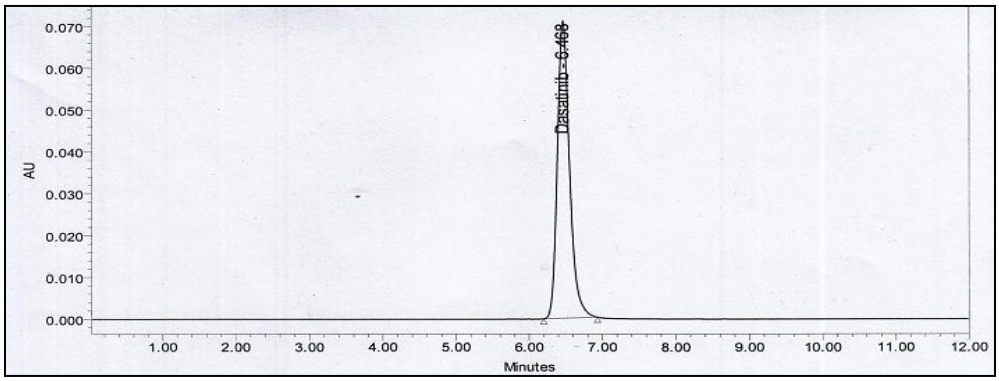

Fig. 3: Typical chromatogram of Dasatinib

Table 1: Optimized chromatographic conditions of Dasatinib

Parameter	Condition
Mobile phase	Buffer: (Acetonitrile:methanol (50:50, v/v)) 50:50, v/v
pH	6.5
Diluent	Mobile phase
Column	Cosmicsil BDS C18 column (150 mm x4.6 mm, 5µ)
Column temperature	Ambient
Wave length	315nm
Injection volume	10 µL
Flow rate	1.0 mL/min
Run time	10 min
Retention time	6.4675 min

Table 2: Linearity results of Dasatinib

Concentration in µg/mL	Area
20	153482
30	228347
40	311353
50	388054
60	460767

Table 3: Recovery results of Dasatinib

Level	Amount added	Amount found	% Recovery	Mean recovery
50%	20.00	20.19	100.95%	100.27%
100%	40.00	40.12	100.30%	
150%	60.00	59.75	99.58%	

Table 4: Precision studies of Dasatinib

Concentration (µg/mL)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
40	0.80	0.74

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

Parameter	Results
Linearity range (µg/mL)	20-60
Correlation coefficient	0.999
Theoretical plates (N)	7620
Tailing factor	1.24
LOD (µg/mL)	0.0405
LOQ (µg/mL)	0.1229

Table 6: Assay results of Dasatinib

Formulation	Label claim	Amount found	%Assay
SPRYCEL®	50 mg	50.02 mg	100.40%

The retention time of Dasatinib was 6.46 min. The number of theoretical plates was 7620 and tailing factor was 1.24 for Dasatinib, which indicates efficient performance of the column. The limit of detection and limit of quantification for Dasatinib were found to be 0.0405 µg/mL and 0.1229 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 100.1% 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, precise, selective and sensitive RP-HPLC method with UV detection 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.

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