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Research Article
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VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DASATINIB IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A new simple accurate and suitable reverse phase high performance liquid chromatographic method was developed for the determination of Dasatinib in bulk and tablet dosage form. The separation was eluted on a Inertsil C_8 column (100 mm x 4.6 mm; 5µ) using a mobile phase mixture of sodium phosphate buffer pH 6.5±0.1 and Methanol in a ratio of 70:30 v/v at a flow rate of 1.0ml/min. The detection was made at 323 nm. The retention times were 5.789±0.1min for Dasatinib. Calibration curve was linear over the concentration range of 5-30µg/ml for Dasatinib. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

KEY WORDS

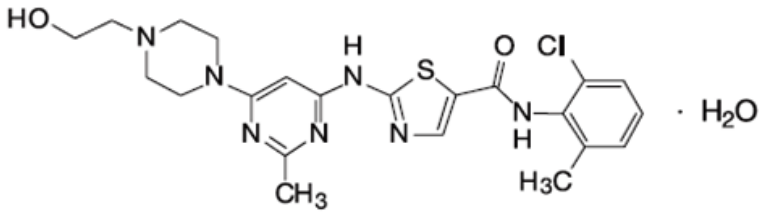
Method development and validation, Dasatinib, Tablets, C_8 column, RP-HPLC.

INTORDUCTION

Dasatinib is an oral medication used for treating chronic myeloid leukemia and acute lymphoblastic leukemia. It is classified as a kinase inhibitor[1]. Kinase inhibitors prevent the growth of tumors by reducing the action of proteins that control cell division, growth, and survival. These proteins are usually present in larger quantities or are more active in cancer cells. By reducing the activity of these proteins, growth and survival of cancer cells are reduced. The chemical name for Dasatinib is N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-thiazolecarboxamide monohydrate (**Figure1**). The molecular formula is $C_{22}H_{26}Cl_lN_7O_2S.H_2O$, which corresponds to a formula weight of 506.02 (monohydrate). The anhydrous free base has a molecular weight of 488.01. Dasatinib is a white to off-white powder and has a melting point of 280°–286°C. The drug substance is insoluble in water and slightly soluble in ethanol and methanol. Dasatinib is an inhibitor of multiple tyrosine kinases[1-2] High-performance liquid chromatographic method for the determination of Dasatinib in rabbit plasma using fluorescence detection and its application to a pharmacokinetic study[3] .Furthermore, dasatinibinhibited the viability of both non-small cell lung cancer and head and neck squamous cell cancer cell lines in vitro through apoptosis-dependent mechanism[4] Literature survey revealed that few analytical methods such as HPLC[5-6], LC-MS[7-8]and UPLC[9] methods have been reported for the estimation of Dasatinib New HPLC–UV Validated Method for Therapeutic Drug Monitoring of Tyrosinekinase Inhibitors in Leukemic Patients[10]. New HPLC–MS method for the simultaneous quantification of theantileukemia drugs Imatinib, Dasatinib, and Nilotinib in human plasma[11]. Dasatinib and Nilotinib are active against most of the Imatinib resistant Bcr–Abl mutants. Imatinib-resistant CML patients who develop resistance against Nilotinib may still show are sponse to Dasatinib, and less frequently, patients with

resistance against Dasatinib may still respond to Nilotinib[12] .Therapeutic Drug Monitoring of the new targeted anticancer agents imatinib,nilotinib, Dasatinib, sunitinib, sorafenib and lapatinib by LC tandem massspectrometry[16]. Simultaneous determination of Nilotinib, Imatinib and its main metabolite.(CGP-74588) in human plasma by ultra-violet high performance liquid chromatography[17].HPLC–MS method for the simultaneous quantification of the antileukemia drugs Imatinib, Dasatinib and Nilotinib in human peripheral blood mononuclear cell[20]. A validated LC–MS/MS assay for the simultaneous determination of theanti-leukemic agent Dasatinib and two pharmacologically active metabolites in human plasma. Application to a clinical pharmacokinetic study[24].liquid chromatographic–mass spectrometric method for the determination of cellular levels of the tyrosinekinase inhibitors lapatinib and dasatinib[26]. Simultaneous measurement of Imatinib, Nilotinib and Dasatinib in dried bloodspot by ultra high performance liquid chromatography tandem mass spectrometry[30].Simultaneous analysis of anticancer agent's bortezomib, Imatinib, Nilotinib, Dasatinib, erlotinib, lapatinib, sorafenib, sunitinib andvandetanib in human plasma using LC/MS/MS[31]. With this present proposed method Dasatinib estimates in tablet formulation.

Figure 1: Structure of Dasatinib



MATERIAL AND METHODS

Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with a Inertsil C_8 column (100 mm x 4.6 mm ; 5µ) and 2489 UV-Visible detector, auto injector, auto sampler with Empower 2 software from Waters corporation, Milford USA was employed in the study. HPLC grade acetonitrile was purchased from Ranbaxy, India, and Sodium dihydrogen phosphate, Sodium Hydroxide, Orthophosphoric acid purchased from SD Fine Chem Mumbai, India were used in the study.

Drug Samples

The reference samples were obtained from M/s. Bio-Leo Analytical Labs India Pvt Ltd, Hyderabad, India, and the formulation samples were purchased from local market.

Mobile phase

Mix an accurately weighed 1.20 gms of Sodium dihydrogen phosphate in 1000ml of water adjust pH 6.5±0.1 with dilute sodium hydroxide solution and acetonitrile in the ratio 70:30 v/v was filtered through 0.45µ membrane filter and was degassed. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered and sonicated by using Bio-Technics India, Mumbai before use. The flow rate of the mobile phase was maintained at 1.0ml/min. The column temperature was maintained at 30ºC and the detection of the drug was carried out at 323nm.

Preparation of stock and working standard solution of Dasatinib

About accurately 20 mg of Dasatinib was weighed accurately on Sartorius semi micro balance model-CPA225D and transfers in to 100ml volumetric flask the solution was sonicated and the resulting solution was diluted with the mobile phase to get a working standard solution of 50 µg/ml. Further dilute 10 ml to 100 ml with mobile phase gives 5 µg/ml.

Sample Preparation

Weighed accurately previously weighed and crushed 20 tablets powder equivalent to 20 mg transferred to 100ml volumetric flask make up to the mark with mobile phase sonicated and filtered through 0.45µ

membrane filter paper. Further dilute 10 ml to 100 ml with mobile phase.

Linearity and Construction of Calibration Curve

Linearity of the peak area response was determined by taking measurement at six concentrations working standard dilution of Dasatinib in the range of 5-30µg/ml. 20µl quantity of the dilution was injected each time in to the column. The drug elutes was monitored at 323 nm at a column temperature of 30^0C and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed.

RESULTS AND DISCUSSION

The present study was aimed at developing a simple precise and accurate HPLC method for the analysis of Dasatinib in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a C_8 stationary phase. A mixture of Sodium Phosphate buffer pH 6.5±0.1: Methanol in a proportion of 70:30 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing, the optical and system suitability parameters are tabulated in **Table 1**. The retention time obtained for Dasatinib was 5.789±0.1 min. Each of the samples was injected and the Sample retention times were observed in all cases. The peak area of Dasatinib was reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed for Dasatinib, The linearity of six different concentrations of overlaid chromatogram of Dasatinib shown in **Figure 2**. The regression concentration and areas are given in **Table 2**. And the regression characters are given in **Figure 4**. When test solutions were analyzed by the proposed method for finding out intra and inter-day variation, low co-efficient of variation was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method are given in **Table 3**.

The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in **Table 4**.The lowest value of LOD and LOQ as obtained by the proposed method by calculated using 3.3xstdev/slope for LOD and 10xstdev/slope for LOQ. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit system suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor and the number theoretical plate are in the acceptable limits.

The system precision was established by six replicate injections of the standard solution containing analytes of interest. The values of relative standard deviation were found within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found within the limit, indicating the injection repeatability of the method. The results were presented in **Table 5 & 6**.

The diluted preparations of marketed tablets were injected in duplicate and the results were calculated and presented in **Table 7**. The chromatogram of sample preparation shown in **Fig 3**.

The specificity of the HPLC method was determined by the complete separation of Dasatinib. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH, Photolytic and Heat degradation. The method does not permit detection of degradation product for Dasatinib. Hence it can be concluded that the proposed HPLC method is accurate, precise, very fast and economical compared to the literature available.

Figure 2: Dasatinib Overlaid linearity chromatogram

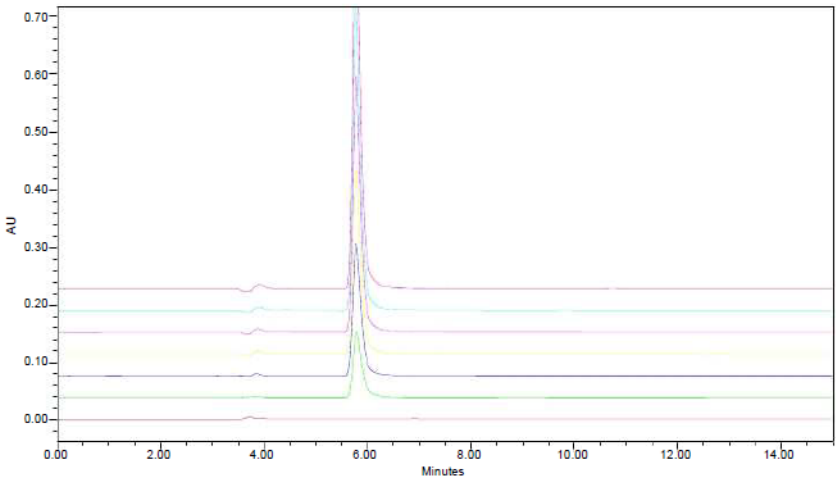


Figure 3: Dasatinib sample chromatogram

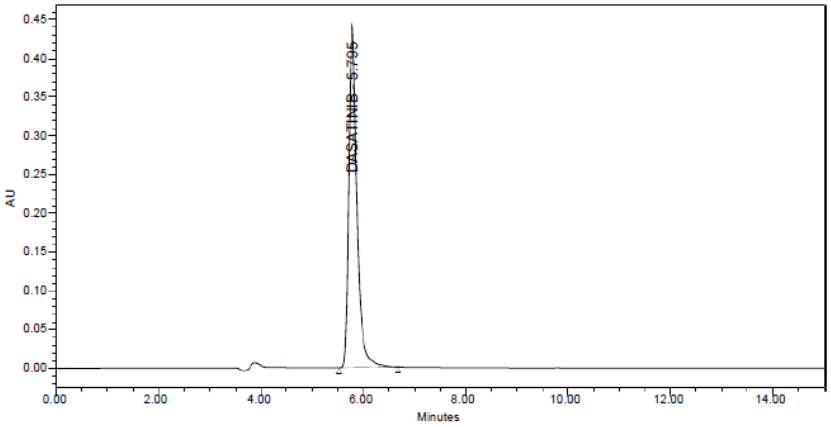


Figure 4: Linearity of Dasatinib

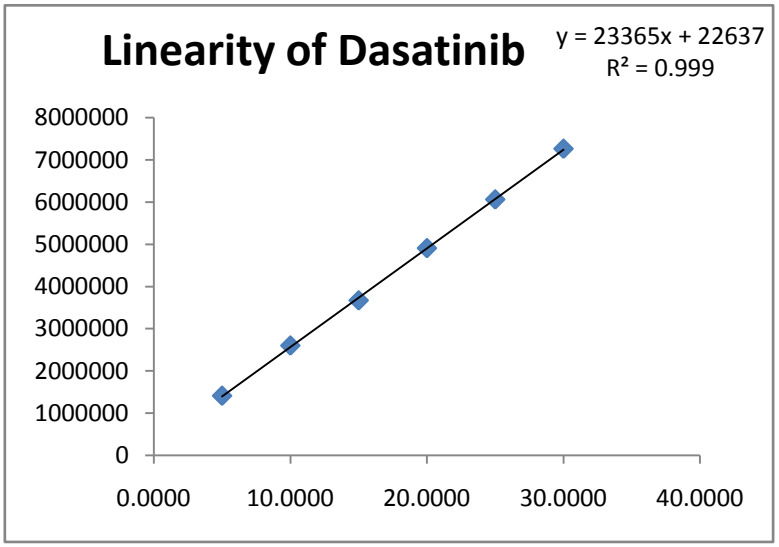


Table 1: Optical and System suitability parameters

Parameter	Dasatinib
Concentration(µg/ml)	5-30
Slope(m)	23365
Correlation Coefficient(r^2)	0.999
Intercept(b)	22637
Symmetry Factor	1.34
Theoretical Plates	7012
LOD(µg/ml)	0.448
LOQ(µg/ml)	1.348

Table 2: Calibration data of the proposed method

Dasatinib Conc (mcg/ml)	Mean Area
5.0000	1406772
10.0000	2596185
15.0000	3666271
20.0000	4904389
25.0000	6058229
30.0000	7259676

Table 3: Accuracy data (Triplicate values at 50,100 &150 percent levels)

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
		2426185	10	9.89	98.90		
1	50%	2421366	10	9.871	98.71	98.78	0.11
		2422044	10	9.873	99.73		
		4902994	20	19.986	99.93		
2	100%	4900765	20	19.978	99.89	99.90	0.02
		4901201	20	19.978	99.89		
		7261453	30	29.601	98.67		
3	150%	7265063	30	29.616	98.72	98.83	0.23
		7292354	30	29.727	99.09		

Table 4: Robustness Study

Parameter	Variation	Retention time(min)	Area	Theoretical Plates	Symmetry Factor
pH	-0.2	5.905	4998506	7004	1.34
	+0.2	5.698	4932454	7024	1.32
	-5^0C	5.781	4912313	7014	1.34
Temperature	$+5^0$C	5.789	4943503	7022	1.33

Table 5: Precision Study

S No	RT	Area
1	5.782	4912014
2	5.785	4910355
3	5.788	4921241
4	5.78	4909632
5	5.789	4905693
6	5.789	4909033
Avg	5.786	4911328
Std Dev	0.004	5283.6
% RSD	0.066	0.11

Table 6: Method Precision study

S No	RT	Area
1	5.778	4902563
2	5.779	4905658
3	5.781	4908978
4	5.783	4915635
5	5.787	4919063
6	5.785	4928362
Avg	5.782	4913377
Std Dev	0.003	9569.3
% RSD	0.060	0.19

Table 7: Assay Results

Drug	Amount present/tablet	Amount Found /tablet	% of Assay
Dasatinib	20 mg	19.931 mg	99.65

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