Research Article

Development and Validation of UV- Spectrophotometric Method for Determination of Dasatinib in Bulk and Pharmaceutical Dosage Form and its Degradation Behaviour Under Various Stress Conditions

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ABSTRACT

A cost effective, precise, accurate, simple stability indicating UV-Spectrophotometric method was developed for the determination of Dasatinib in bulk and tablet dosage form. Dasatinib shows highest λ_{max} at 323 nm. Beer's law was found over a concentration range of 2-10 µg/ml with superior correlation coefficient (r^2 = 0.999). The Detection limit (DL) & Quantitation limit (QL) were found to be 0.3968 µg/ml and 1.2025 µg/ml respectively. The results of the Dasatinib recovery analysis were found to be 99.9505 ± 0.0002 to 100.0645 ± 0.0002. Percentage assay of Dasatinib tablets (Dasanat) got more than 99.88 %. Dasatinib was subjected to alkali, acid, oxidation, thermal, UV light degradation. Dasatinib is more unstable in acidic, oxidation, thermal and stable in alkaline and ultra violet (UV) light irradiation. The Proposed spectrophotometric method was validated as per the ICH Q1A (R2) guidelines. While estimating the Dasatinib in tablet formulation there was no interference of additives & excipients. Hence this method can safely be employed for the routine quality control analysis of Dasatinib in bulk and tablet dosage form.

Keywords: Dasatinib, Method development, UV spectroscopy, Forced degradation.

INTRODUCTION

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. Dasatinib is utilized for the treatment of chronic myeloid leukemia and acute lymphoblastic leukemia.

From the extensive literature survey reveals that not many UV analytical methods published to describe the quantification of Dasatinib by LC-MS/MS[1-4], LC-MS[5-6], HPTLC[7-8], UPLC[9], HPLC-MS[10], RP-HPLC[11], UV-Spectrophotometric method[12]. Infact the published UV method utilizes only methanol as solvent. But there is no stability indicating UV method with acetonitrile and methanol as solvent hitherto. These stability indicating methods would be helpful in establishing the stability data of these drugs in bulk and tablet dosage forms. Generally this UV technique is less expensive and with inherent simplicity. Rapid development in the pharmaceutical industries, producing more number of new drugs and formulations in different parts of world has been increasing. For providing effective and safe drug formulation to consumers direly needed. So innovative new analytical methods compulsory for controlling their quality and amount of drug in pharmaceutical dosage forms particularly it plays an essential role in the case of powerful drugs. So the author inclined to select a new, simple and fast stability indicating UV spectrophotometric analytical method to quantify Dasatinib in bulk and tablet dosage forms. Figure 1 illustrates the structure of Dasatinib.

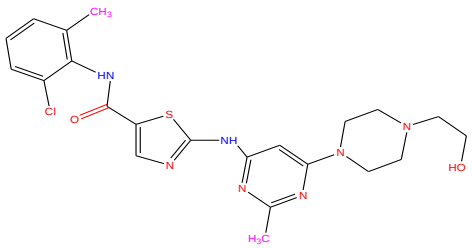

Figure 1: Chemical structure of Dasatinib

MATERIALS AND METHODS

Instruments used ELICO Double beam SL 210 Ultra violet-Visible spectrophotometer consisting two matched quartz cells with one cm light path was utilized for measuring of absorbance of Dasatinib. Essaevibra AJ 0.001 g balance was utilized for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai were utilized in this study.

Materials

Dasatinib pure drug was procured as gift sample by Hetero Drugs Ltd., Hyd, Telangana, India. The Dasanat tablets containing 20mg of Dasatinib procured from local market. Acetonitrile and methanol were procured from E. Merck specialties private Ltd., Mumbai, India.

Selection of solvent

Copious trails were done to find out the right solvent system for dissolving the drug. The solvents like acetonitrile, methanol, double distilled water and dimethyl sulfoxide [DMSO] were tried depending on the solubility of the Dasatinib. Dasatinib is soluble in organic solvents such as acetonitrile, methanol and DMSO. Insoluble in distilled water. Based on the solubility

methanol and acetonitrile were selected all the way through the experiment.

Selection of detection wavelength

To determine the optimum λmax, Dasatinib 10 µg/ml of working standard solution was prepared and scanned in UV wavelength range of 200-400 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 323 nm which was chosen as the detection wavelength for the estimation of Dasatinib.

Preparation of stock and working standard solution

Dasatinib 10 µg/ml standard stock solution was done by transferring precisely weighed 10 mg of standard Dasatinib to 10 ml volumetric flask and dissolved in methanol and acetonitrile. The volume was filled upto the mark with methanol and acetonitrile. From this solution 1 ml was precisely transferred into a 10 ml volumetric flask and volume was made up to the mark with methanol and acetonitrile. Again from the above solution 1 ml was precisely transferred into a 10 ml volumetric flask and volume was made upto the mark with methanol and acetonitrile. Working standard solutions of Dasatinib was prepared by suitable dilution of the stock solution (10 µg/ml) with the methanol and acetonitrile.

Preparation of Calibration curve

A calibration curve was plotted over a concentration range of 2-10 µg/ml for Dasatinib. Precisely measured standard solution of Dasatinib (2, 4, 6, 8, and 10 ml) was shifted to a series of 10 ml volumetric flasks and the volume was filled upto 10 ml with methanol and acetonitrile. Calibration curve was done by plotting Dasatinib concentration on X-axis and their respective absorbances on Y-axis. Calibration data is shown in table 1. The optical characteristics are shown in table 2. Figure 2 shows the overlain spectrum of Dasatinib. The calibration curve is exhibit in figure 3.

Table 1: Calibration data of Dasatinib

S.No	Concentration (µg/ml)	Absorbance (nm)
1	2	0.2079
2	4	0.4058
3	6	0.6197
4	8	0.8468
5	10	1.0761

Forced Degradation Studies

To evaluate the stability indicating property of the proposed UV method stress studies were done under ICH[13-16] recommended conditions. Forced degradation of Dasatinib was carried out by exposing the bulk sample to alkaline, acidic, oxidative, dry heat and neutral conditions.

Irradiation with UV light

A sample powder of Dasatinib (10 mg) was exposed to UV light for 48 hours. The material was dissolved in 10 ml methanol and acetonitrile. The solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted and absorbance was recorded and shown in figure 4a.

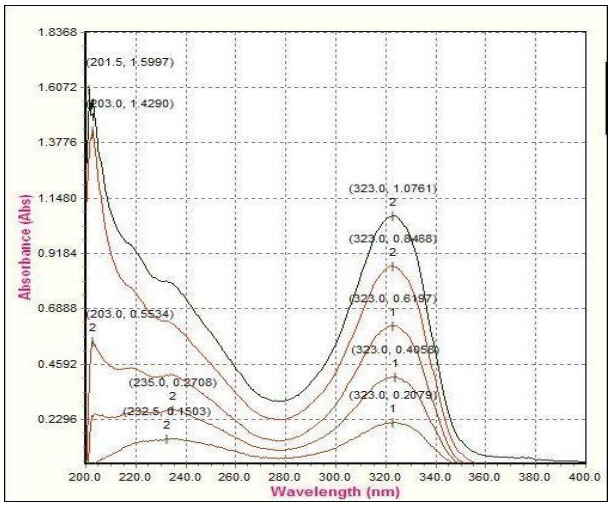

Figure 2: Overlay Spectrum of Dasatinib

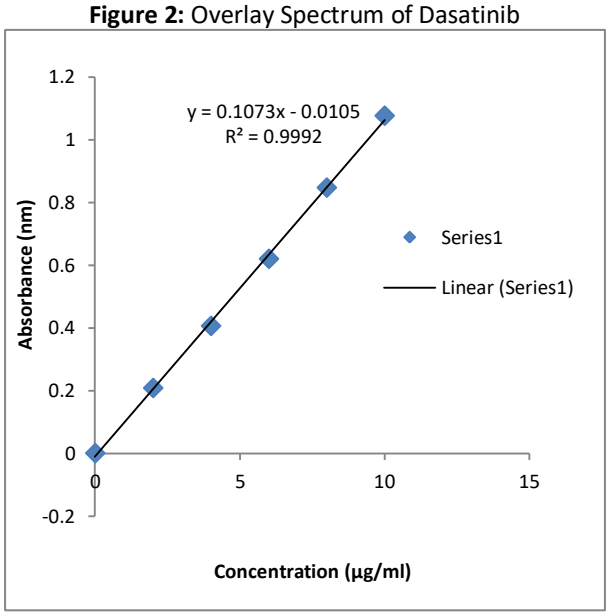

Figure 3: Calibration curve of Dasatinib

Figure 4a: Irridation with UV light

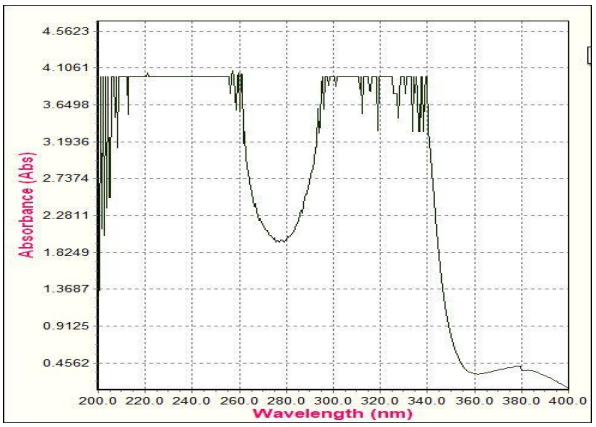

Figure 4b: Thermal degradation

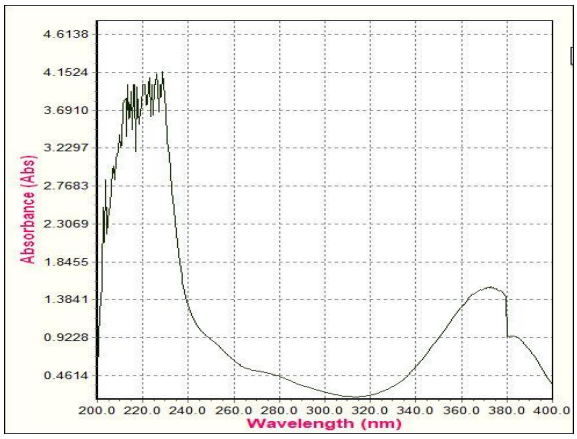

Figure 4c: Oxidative degradation

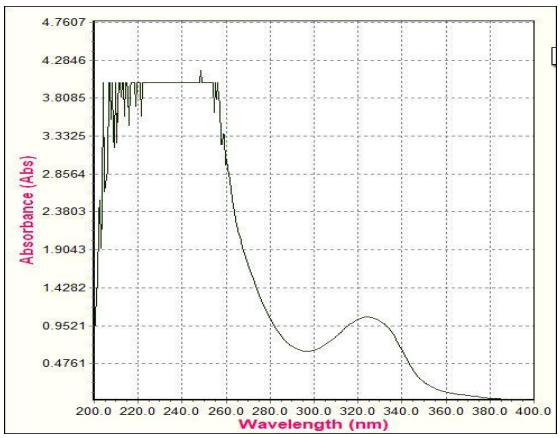

Figure 4d: Alkaline degradation

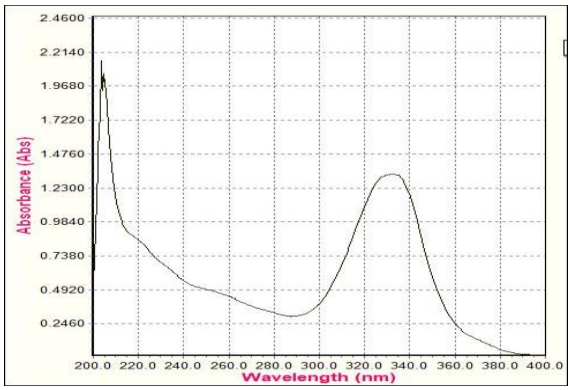

Figure 4e: Acidic degradation

Thermal degradation

A sample powder of Dasatinib (10 mg) was exposed to a temperature of 70ºC for 48 hours in hot air oven. The drug was dissolved in 10 ml methanol and acetonitrile. The drug solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was diluted and absorbance was recorded. Figure 4b shows the thermal degradation study.

Oxidative degradation

Exactly 10 µg/ml of Dasatinib solution was taken into round bottom flask. The contents were then mixed with 9 ml of 30 % hydrogen peroxide solution, and the contents were allowed to proceed at room temperature (25°C) for 2 hours with intermittent shaking and absorbance was recorded which is shown in figure 4c.

Alkaline and Acidic hydrolysis

Aliquot of 10 µg/ ml of Dasatinib solution was transferred to a small round bottom flask. The solution was mixed with 9 ml of 1 M hydrochloric acid or 1 M sodium hydroxide. The prepared solutions were subjected to reflux for 2 hours in a boiling water bath. The samples were cool-down to room temperature (25°C), and absorbance was recorded as shown in figures 4d and 4e.

RESULTS AND DISCUSSION

Method development and Validation

Several solvents were analysed including acetonitrile, DMSO, and methanol at 10 µg/ml concentrations. However Dasatinib was soluble and stable for minimum 48 hours at room temperature. So methanol and acetonitrile solvent was utilized for the estimation of detection wavelength and preparation of standard and working concentration. In order to check the proposed method to the pharmaceutical formulation, an assay of Dasanat 20 mg tablets was used at working concentration. Assay for working concentration of sample at 323 nm was in limits of acceptance 98-102 %. According to ICH Q1A (R2) has provided guidelines for validation of analytical method which defines this process as characteristic performance that is established by laboratory studies. UV spectrophotometric method developed according to guidelines for validation of analytical procedures. The method was validated for parameters such linearity, precision, accuracy, specificity, robustness, ruggedness, DL and QL.

Precision

In system precision 10 µg/ml concentrations of 6 replicate recordings of absorbance at 323 nm were observed on the same day and corresponding responding responses were evaluated. The mean, SD and % RSD were calculated. The repeatability data of Dasatinib is summarized.

Table 2: Summary of Optical characteristics and validation parameters

S.No	Parameters	Result
1	Detection wavelength	323 nm
2	Beer's Law limits (µg/ml)	2-10 (µg/ml)
3	Regression equation (y = mx+c)	0.107x - 0.010
4	Correlation Coefficient (r^2)	0.999
5	Slope (m)	0.107
6	Intercept (c)	0.010
7	Precision (% RSD) System precision Intra-day (n = 9) Inter-day (n = 9)	 0.0510 0.0245 - 0.0512 0.0245 - 0.0512
8	Accuracy (% mean recovery) 80 % level 100 % level 120 % level	 100.0082 100.0269 99.9999
9	Ruggedness 2 Analyst (% RSD) 2 instruments (% RSD)	 ≤ 2
10	Robustness Wavelength (± 2nm) (% RSD)	 ≤ 2
11	LOD and LOQ	0.3968 and 1.2025

The intermediate precision or inter-day precision is evaluated by analyzing 10 µg/ml concentrations of Dasatinib fresh sample solutions were analyzed six times on 3 different days and was evaluated. Eventually the mean, SD and % Relative standard deviation were counted.

Accuracy (Recovery studies)

Recovery studies of Dasatinib were carried out by utilizing standard addition method. By preparing the known amount of standard Dasatinib drug at 3 levels (80 %, 100 % and 120 %) was added to pre-analyzed sample and again re-analyzed by duly adopting the present developing method. Infact, from the amount of Dasatinib found, % recovery was estimated.

Ruggedness

Ruggedness is determined by different analysts, instruments, laboratories. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. Suggested % RSD less than 2 and indicates that the method developed is rugged.

Robustness

The prominent part of robustness is to develop methods that allow for predictable variations in the separation parameters. For the estimation of method robustness, parameters such as variation in detector wavelength are varied within the accurate range and the quantitative influence of the variables is determined. The analysis showed % RSD less than 2 which indicates that the method established is robust.

LOD and LOQ

Limit of Detection and Limit of Quantification were calculated using following formula LOD = 3.3 * (SD) / S and LOQ = 10 * (SD) / S, where SD = standard deviation of absorbance and S = slope of the calibration.

Procedure for assay of pharmaceutical formulation

20 Dasatinib (Dasanat) marketed tablets were accurately weighed, finely powdered and average weight of each tablet was determined and the tablet fine powder equivalent to 10 mg of Dasatinib was taken into 100 ml graduated flask and dissolved in methanol and ACN (acetonitrile) to get 100 µg/ml concentration. The solution was then sonicated for 20 min and filtered and further dilutions were done with ACN to get eventual concentration (10 µg/ml) within the linearity range and measured λ_{max} at 323 nm. Finally the drug content in each tablet and also bulk drug was found by utilizing the standard graph.

For analysis of bulk drug

10 mg of bulk drug was accurately weighed in 10 ml volumetric flask, 4 ml of ACN and 6 ml of methanol was added to get the drug soluble and eventually the volume was filled upto 10 ml and required concentration (10 µg/ml) was prepared and determined the absorbance. Table 3 shows the assay results of pharmaceutical formulation (Dasanat) and bulk drug.

Table 3: Result of Assay of Pharmaceutical Formulation (Dasanat) and bulk drug

Pharmaceutical formulation and pure drug	Absorbance ± S.D.	% RSD	% Recovery* (Amount found)
Dasanat	0.6190± 0.4877	0.4896	99.8870
Bulk drug	0.6193± 0.0672	0.0672	100.0645

*mean of six determinations

Summary

The UV spectrum of Dasatinib was scanned in the region between 200-400 nm. The overlay spectra of Dasatinib at different concentrations (figure 2) were appreciably absorbed maximum at 323 which was selected as the detection wavelength. The response of the Dasatinib was found to be linear in the ranges from 2-10 µg/ml with a good correlation coefficient of r^2 = 0.999 and the Figure 3

shows the Dasatinib linearity calibration curve and the table 1 shows the calibration data. Optical characteristics and validation parameters of the proposed analytical method are represented in table 2. It was found that the % RSD values of intra-day and inter-day precision was 0.0324 and 0.0274 respectively pertaining to Dasatinib and the values of % RSD [<2.0] pellucidly showed that the method was fairly precise. According to norms in vogue accuracy studies were conducted by recovery study using standard addition method at 3 different concentration levels (80, 100 and 120 %). The recovery study results were found to be within the limits and % RSD less than 2. Ruggedness was performed by changing two different analysts and two different instruments and % RSD was calculated. The % RSD clearly shows less than 2 which indicate that the method was rugged. Robustness was performed by changing two different wavelengths. Even though by changing the minor modifications the % RSD got < 2 which shows that the method was robust. In the planned method the LOD and LOQ were found to be 0.3968 µg/ml and 1.2025 µg/ml respectively which show that this method was very sensitive as they were within the permitted levels. The developed method was eventually utilized for quantification of tablet dosage form and bulk form. The mean % assay value for pure powder and tablet formulation were found to be 100.0645 ± 0.0002 and 99.8870 ± 0.0002 respectively. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method. Forced degradation studies were done. Extensive degradation studies found in oxidation but very little degradation found in acidic, stable to UV and alkaline and completely degraded in thermal.

CONCLUSION

There were no UV methods have been reported (with methanol and acetonitrile as solvent) for the determination of Dasatinib in bulk as well as pharmaceutical tablet. None of the usual excipients employed in the formulation of Dasatinib dosage forms interfered in the analysis of Dasatinib by the developed method. Validation parameters are found within the limits. As a matter of fact, in this study the degradation behaviour of Dasatinib was studied by subjecting the Dasatinib to different stress conditions as per ICH guidelines. The additional findings in this study show that the drug undergoes an extensive degradation under oxidation, slight degradation in acidic, stable in UV and alkaline and completely degraded in thermal. It was observed that all the statistical analysis results of % RSD values particularly precision, accuracy are observed below two which speaks that the method is precise and accurate. The results of pharmaceutical formulation asserts that the proposed method of Dasatinib suitably practicable for their determination without interfering the additives and excipients. Therefore this method was simple, precise, accurate and cost effective and in actual fact possible for routine sample analysis of Dasatinib in bulk and pharmaceutical tablets.

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