Chemical Concepts

International Journal of Chemical Concepts
ISSN:2395-4256 www.chemconsai.com
Vol.01, No.01, pp 26-33, 2015

Spectrophotometric Method for the Determination of Dasatinib in Pharmaceutical Formulations and Human Blood samples with BPB

B. Ramachandra, K.Sivarami Reddy, T. Niranjan, & N.Venkatasubba Naidu*

Department of Chemistry, S.V.University, Tirupati -517502, A.P., India.

Abstract: A simple, sensitive, selective rapid spectrophotometric method has been developed for the determination of dasatinib in pure form and pharmaceutical formulations based on the ionissotiation reaction with BPB reagent, at P^H–3.9 which is extractable at 420 nm. Beer's law is obeyed in the concentration ranges 5-30 µg ml^{-1} and 3-18µg $ml^{-}1$ for blood sample the developed method was applied directly and easily for the analysis of the Pharmaceutical formulations and blood sample. R.S.D was found to be 0.32679%, 0.54644% and Recovery 99.86% 99.9% respectively. The method was completely validated and proven to be rugged. The interferences of the other ingredients and excipients were not observed. The repeatability and the performance of the proved method were established by point and internal hypothesis and through recovery studie**S.**

Keywords: Spectrophotometry, Dasatinib, Blood Sample & BPB / $Chcl_3$.

Introduction

Dasatinib (Fig. 1) is chemically N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-thiazolecarboxamide monohydrate. It is a tyrosine kinase inhibitor[1] and is used in patients with chronic myelogenus leukemia after Imatinib treatment and Philadelphia chromosome positive acute lymphoblastic leukemia[2] Dasatinib is a second-generation inhibitor of the BCR-ABL and SRC tyrosine kinases. In vitro, dasatinib inhibits the BCR-ABL kinase with 325-fold greater potency than Imatinib[3]. In addition, it showed significant activity in Phase II studies in Philadelphia-positive acute lymphoid leukemia (Ph + ALL) patients who were resistant or intolerant to imatinib[4]. In pharmacokinetic studies, dasatinib exposure was shown to vary linearly and proportionally with dose. Dasatinib has in vitro activity against all imatinibresistantBCR-ABL mutations, with the notable exception of T315I[1]. For example, a previous study reported that12 of 17 relapsed Ph +ALL patients acquired a T315I mutation during dasatinib therapy[3]. Additionally, there is little data on the relationship between plasma dasatinib concentration and outcome or adverse events, and no clinically relevant data to suggest that dose changes are necessary based on sex, age, or pharmacokinetic variation ofdasatinib transporters. Moreover, little is known about the relationship between dasatinib pharmacokinetics and the emergence of BCR-ABL kinase domain mutations in vivo. To determine whether plasma dasatinib pharmacokinetics influences BCR-ABL mutations, we used high-performance liquid chromatography (HPLC) to measure the plasma dasatinib concentrations in Ph + ALL patients undergoing dasatinib monotherapy. Literature survey reveals that, onechromatographic[3] (HPLC, HPTLC) method has been reported for the estimation of dasatinib. No UV method was reported in literature for analysis of dasatinib. Hence, an attempt has been made to develop a new UV spectrophotometric method for estimation of dasatinib in Blood sample, bulk drugs and pharmaceutical formulations.

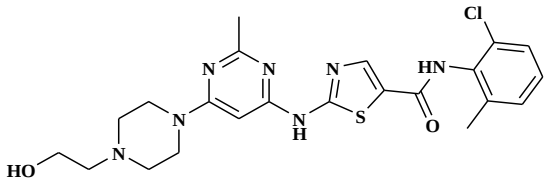

Figure-1 Chemical structure of Dasatinib

All spectral and absorbance measurements were made on a Shimazdu model 2450 digital spectrophotometer with 10 mm matched quartz cells.

Experimental

Preparation of Standard calibration curve of pure drug.

Solvent

Methanol was used as Solvent.

Preparation of Calibration curve

Fresh aliquots of Dasatinib ranging from 0.5 to 3.0 ml and 2 ml of 0.2 % Bromophenol Blue (BPB), 1ml (0.5N) Hydrochloric acid solution and 2ml of chloroform was added was added the final concentration range of 5 to 30 µg ml^{-1} were transferred into a 50 ml separating funnel the solutions in funnel were shaken for 2min.The two phases were allowed to separate and the absorbance of yellow colored chromogen was measured at 420 nm against reagent blank. The color species was stable for 32 h. The amount of Dasatinib present in the sample solution was computed from its calibration curve.

Procedure for formulations

Twenty tablets containing Dasatinib were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Dasatinib was dissolved in a 100 ml of Methanol and mixed for about 5 min and then filtered. The Methanol was evaporated to dryness. The remaining portion of solution was diluted in a 100 ml volumetric flask to the volume with Methanol up to 100 ml to get the stock solution A. 10 ml of aliquots was pipette out into 100 ml volumetric flask and the volume was made up to the mark with Methanol to obtained the final concentration of 100µg ml^{-1} (Stock solution). Subsequent dilutions of this solution were made with Methanol to get concentration of 5 to 30 µg ml^{-1} and were prepared as above and analyzed at the selected wavelength, 420 nm and the results were statistically validated.

Procedure for Blood sample

After collection of Blood sample it will be centrifuged. For isolation of Dasatinib from plasma sample, Methanol was used for protein precipitation. Liquid- Liquid extraction was performed with plasma by alkalinization with 1M NaOH, followed by extraction with 30% dichloromethane in Hexane. The upper organic layer was evaporated to dryness, the dry residue 100 mg was dissolved in 100 ml of Methanol (1000 µg ml^{-1}). From the above solution 10 ml is taken into a 100 ml of Volumetric flask and made up to the mark with Methanol (100 µg ml^{-1}) from the above solution ranging from 0.3.-1.8 ml and 2 ml of 0.2% Bromophenol Blue (BPB), 1ml (0.5N) Hydrochloric acid solution and 2ml of chloroform was added was added the final concentration range of 3- 18µg ml^{-1} were transferred in to 50 ml separating funnel the solutions in funnel were shaken for 2min.The two phases were allowed to separate and the absorbance of yellow colored chromogen was measured at 420 nm against the reagent blank. The color species was stable for 32h. The amount of Dasatinib present in the sample solution was computed from its calibration curve.

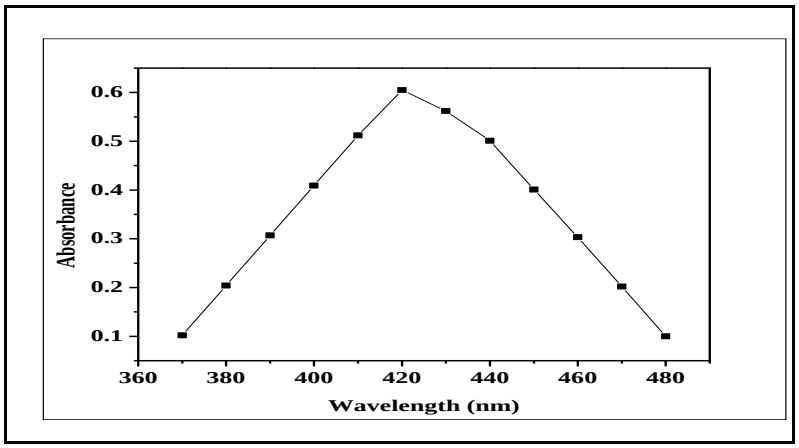

Fig-2: Absorption spectrum of Dasatinib with BPB /$CHCl_3$

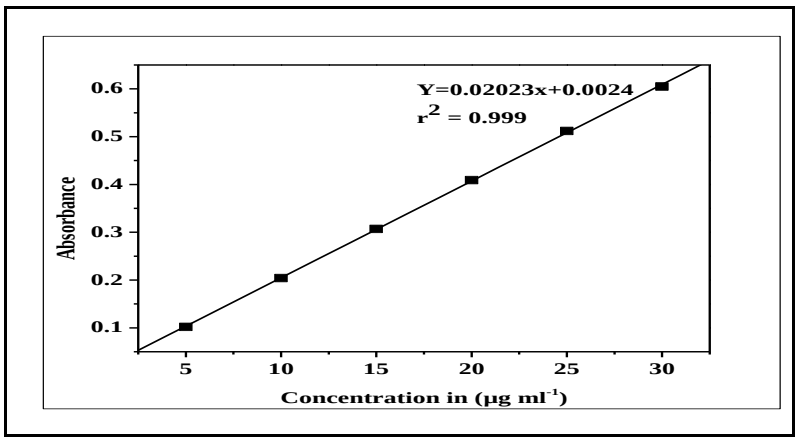

Fig-3: Beer's law plot of Dasatinib with BPB /$CHCl_3$

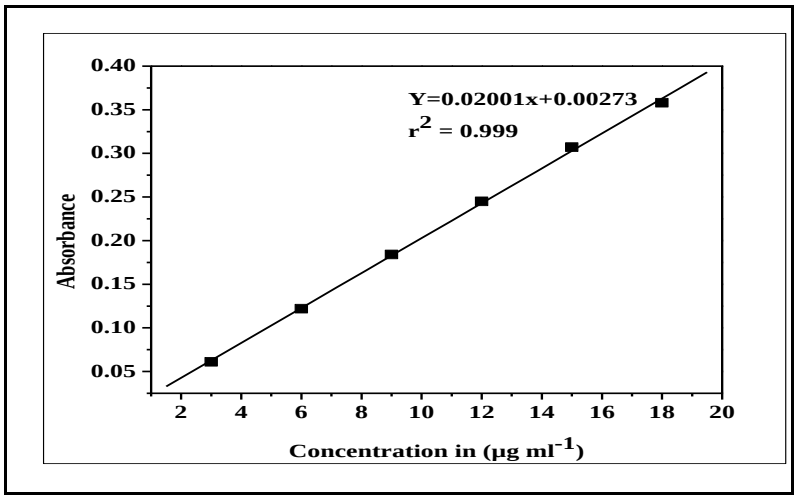

Fig-4: Beer's law plot for BPB in Blood sample

$$\text{Dasatinib} + \text{CHCl}_3 + \text{HCl} \xrightarrow[\text{Ion association reaction}]{\text{BPB}} \text{Yellow cloured species}$$

Fig-5: A Schematic reaction Mechanism of Dasatinib with BPB

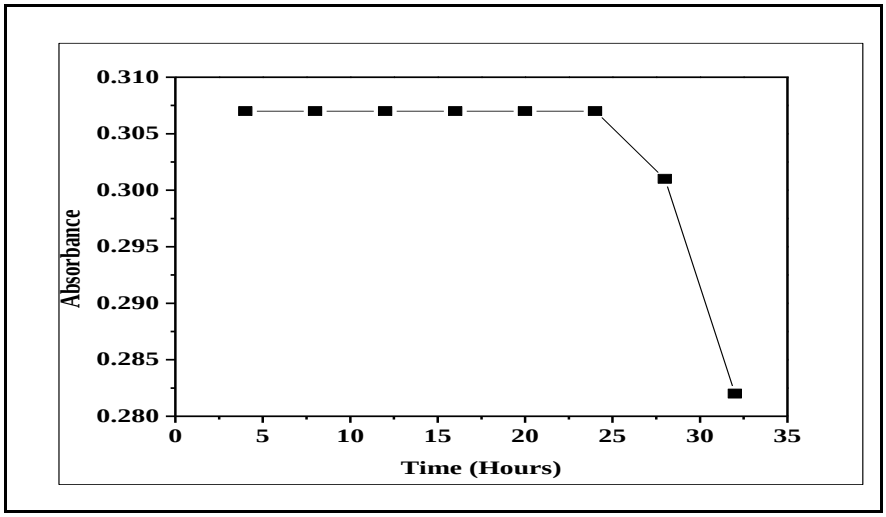

Fig-6: Color stability data for BPB Method

Table-1. Optical characteristics and precision by BPB

Parameter	Visible method
Color	Yellow
Absorption maxima (nm)	420
Beer's law limits (µg ml^{-1})	5-30
Molar absorptivity (l $mol^{-1}cm^{-1}$)	$0.02016x10^4$
Sandell's Sensitivity (µg cm^{-2})	1.65289
Regression equation (Y*)	Y=mx+c
Slope (b)	0.02023
Intercept(a)	0.0024
Standard deviation(SD)	0.00322
Correlation coefficient (r^2)	0.999
%RSD (Relative Standard deviation)	0.09593
Limits of detection (LOD)(µg ml^{-1})	0.47750
Limits of quantification (LOQ) (µg ml^{-1})	1.59619

RSD of 6 independent determinations

Table-2. Assay results of Dasatinib in formulations by visible Method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method[31,32] (mg)	% Recovery
IMATINIB	250	249.97 T=0.002969 F=3.55826	249.20	99.97
NILOTINIB	250	249.94 T=0.002968 F=3.55776	248.57	99.94

- **T and F- values refer to comparison of the proposed method with reference method.**
- **Theoretical values at 95% confidence limits t= 0.00297 and F= 2.6177**

Table-3. Determination of accuracy of Dasatinib

Amount of DSB in formulation (mg)	Amount of Standard DSB added (mg)	Total amount found (mg)	% Recovery
249.79	200	449.62	99.62
249.49	200	449.08	99.08
249.03	200	448.25	98.25
249.93	250	499.86	99.86
249.84	250	499.68	99.68
249.77	250	499.54	99.54
249.49	300	548.87	98.78
249.39	300	548.65	98.65
249.28	300	548.41	98.41

Table-4. Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
448.98	0.6901	0.15370
499.69	0.16042	0.03210
548.61	0.23007	0.04193

The results are the mean of three readings at each level of recovery.

Table-5 Repeatability data for DSB at 420 nm BPB

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%)RSD
5	0.102	0.101	0.102	0.10167	0.00058	0.57047
10	0.204	0.203	0.202	0.203	0.001	0.49261
15	0.307	0.305	0.306	0.306	0.001	0.32679
20	0.409	0.407	0.408	0.408	0.001	0.24509
25	0.504	0.503	0.502	0.503	0.001	0.19880
30	0.605	0.604	0.605	0.6046	0.00058	0.09593

Average of six determinations.

Table-6. Color stability data for BPB Method

Conc. inµg ml^{-1}	Time in Hours							
15	4	8	12	16	20	24	28	32
	0.307	0.307	0.307	0.307	0.307	0.307	0.301	0.282

Table-7. Assay results of Dasatinib in Blood sample

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method in (mg)	Amount found by the reference method[31,32] (mg)	% of Recovery
IMATINIB	2	1.95 T=0.00297 F=3.4871	1.87	99.95
NILOTINIB	2	1.85 T=0.00296 F=3.4218	1.89	99.85

- **Tand F values refer to comparison of the proposed method with reference method.**
- **Theoretical values at 95% confidence limits t=0.00195 and F=2.1384.**

Table-8 Determination of accuracy of Dasatinib

Name of the Formulation in (mg)	Amount of Drug in Blood sample(mg)	Amount of Standard Drug added in (mg)	Total amount found(mg)	% Recovery
2	1.95	2	3.9	99.9
2	1.85	2	3.7	99.7

The results are the mean of two readings at each level of recovery.

Table-9 Repeatability data for Dasatinib at 420nm

Concentration in µg ml^{-1}	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
3	0.061	0.060	0.059	0.060	0.001	1.66666
6	0.122	0.122	0.120	0.1213	0.00115	0.94806
9	0.184	0.182	0.183	0.183	0.001	0.54644
12	0.245	0.243	0.244	0.244	0.001	0.40983
15	0.307	0.306	0.305	0.306	0.001	0.32679
18	0.358	0.356	0.358	0.3573	0.00115	0.32185

Average of six determinations

Results and Discussions

Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV Spectrophotometric method (Reference method – A) and of the colored species formed in each so the visible spectrophotometric method, specified amount of Dasatinib in solution 5-30 µg ml^{-1} were taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 200-400 nm (for method A) and 200-800 nm (for method B) against corresponding reagent blank. The regent blank absorption spectrum of each method was also recorded against distilled water /Methanol. The results are graphically represented in (fig- 2).

Parameters fixation

In developing these methods, a systematic study of the effects of various relevant parameters in the method concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development with BPB Method reproducibility and reasonable period of stability of final colored species formed. The following studies were conducted.

Method

The results obtained in this method were based on ion association reaction of Dasatinib with BPB chloroform and distilled water to form yellow colored chromogen that exhibited maximum absorption at 420 nm against the corresponding reagent blank. The functional group used for the color development for this method was hydroxyl group. A schematic reaction mechanism of Dasatinib with acidic dye BPB reagent was shown in (fig-5). The effect of various parameters such as concentration and volume of BPB and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameters at a time.

Optical Characteristics

The reference method adhere to beer's law the absorbance at appropriate wave length of a set of solutions contains different amounts of Dasatinib and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank. The beers law plot of the system illustrated graphically least square regression analysis was carried out for the slope intercept and Correlation Coefficient. Beer's law limits, Molar absorptivity & Sandell's sensitivity for Dasatinib with each of mentioned

reagents was calculated. In order to test whether the colored species formed in the method adhere the beer's law the absorbance at appropriate wavelength of a set of solutions contain different amounts of Dasatinib and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The beers law plots of the system illustrated graphically (fig-3&2) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity Sandell's sensitivity for Dasatinib with each of mentioned reagents were calculated. The optical characteristics are presented in the table –1.

Precision

The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtain by actual determination of a fixed amount of Dasatinib n 5, 10& 15 µg ml^{-1} in final solution. The percent relative standard deviation were calculated for the proposed methods and presented in tables –1.

Analysis of formulations

Commercial formulations of Dasatinib were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the T and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in table-2. The proposed methods also applied for Biological Samples (Blood) for good recoveries are obtained which were recorded in table-7.

Accuracy

Recovery studies were carried by applying the Standard addition method to Drugs sample present in formulations for the known amount of Dasatinib the recovery studies were carried .By applying the same method to Biological sample (Blood) to which known amount of Dasatinib corresponds to 2 mg Formulations taken by the patient. By the follow of Standard addition method 2 mg of label claim was added.After the addition of these standards the contents were transferred to 100 ml volumetric flash and dissolved in solvent. Finally the volume was made up to the mark with solvent. The solution was filtered through Whitman No. 41filter paper. The mixed sample solutions were analyzed and their absorbance value was determined. At each level of recovery five determinations were performed and present in Table–3. The results obtain were compared with expected results and were statistically validated in Table–4.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

Specificity and Selectivity

Specificity is a procedure to detect quantitatively analyze in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyze qualitatively in presence of components that may be expected to present in the sample matrix. The excipients in formulations were spiked in a pre weighed quantity of Drugs and then absorbance was measured and calculations were done to determine the quantity of the Drugs.

Repeatability

Standard solutions of Dasatinib were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure six times and standard deviation was calculated and presented in tables -5&9.

Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 32 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in Table-6.

Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Dasatinib under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in formulations.

Conclusion

The proposed method was found to be simple, economical and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of blood samples and formulation containing dasatinib showed no interference from common excipients. Hence this method could be considered for the determination of dasatinib in quality control laboratories.

Acknowledgements

The authors are grateful to S.V.University for providing the laboratory Facilities and to UGC-BSR., New Delhi for providing financial assistance.

References

1. J. Das, *J. Med. Chem.*, 49, 6819 (2006).
2. M. Talpaz, N. Shah, H. Kantarjian, N. Donato, J. Nicoll, R. Paquette, J. Cortes, S. O'Brien,C. Nicaise, E. Bleickardt, M. Blackwood-Chirchir, V. Iyer, T. Chen, F. Huang, A. Decillis andC. Sawyers, *N. Engl. J Med.*, 354, 2531 (2006).
3. O'Hare T, Walters DK, Stoffregen EP, Jia T,Manley PW, Mestan J, Cowan-JacobSW, LeeFY, Heinrich MC, Deininger MW, Druker BJ: In vitro activity of Bcr-Ablinhibitors AMN107 and BMS-354825 against clinically relevant imatinibresistant Abl kinase domain mutants. Cancer Res 2005, 65:4500–4505.
4. Ottmann O, Dombret H, Martinelli G, Simonsson B, Guilhot F, Larson RA,Rege-Cambrin G, Radich J, Hochhaus A, Apanovitch AM, et al: Dasatinib induces rapid hematologic andcytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood 2007, 110:2309–2315.
5. Foa R, Vitale A, Vignetti M, Meloni G, Guarini A, De Propris MS, Elia L,Paoloni F, FaziP, Cimino G, et al: Dasatinib as first-line treatment for adultpatients with Philadelphia hromosome-positiveacute lymphoblasticleukemia. Blood 2011, 118:6521–6528.
6. D.V. Mhaske and S.R. Dhaneshwar, *Chromatographia*, 66, 95 (2007).

***** ***** *****