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Research Article

DEVELOPMENT OF COLORIMETRIC METHOD FOR DETERMINATION OF DASATINIB IN BULK AND IN TABLET FORMULATION

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ABSTRACT

A simple, economical, accurate, precise and reproducible colorimetric method for the routine estimation of dasatinib has been developed. The method is based on the formation of a blue colored complex by dasatinib in presence of folin ciocalteu reagent and NaOH. The developed colored complex showed λmax at 745 nm. Beer's law in the concentration range of 10 to 80 µg/ ml. Results of analysis were authenticated statistically as well as by recovery studies, which gave mean recovery between 99 to 100%. The method was successful in determining dasatinib in physical mixture and in tablet formulation, with an average recovery of 99 and 100 % respectively. The proposed method could find application to product development scientists in ongoing research; as well provide an additional tool for routine analysis of dasatinib in academia and quality control laboratory.

Keywords: Dasatinib, Colorimetric, Method validation, Bulk and tablet formulation.

INTRODUCTION

Dasatinib, N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-thiazole carbox-amide monohydrate is a novel, oral multi-targeted inhibitor of kinases including SRC family kinases. Dasatinib (DTB) is highly potent and has demonstrated in vivo anti-tumor activity in several human tumor xenograft models[1, 2]. It is used for the treatment of imatinib-resistant chronic myeloid leukemia[3, 4]. Dasatinib was the first agent approved to treat patients with CML who are intolerant or resistant to imatinib.

Literature survey serves only HPLC[5], HPTLC[5] and simple spectrophotometric method[6] for analytical estimation of DTB; however, no spectroscopic-colorimetric studies for its estimation have been reported till date. Hence it was thought worthwhile to develop spectrophotometric-colorimetric method for the same. In the present study, a colorimetric method for the determination of DTB in bulk and in its pharmaceutical formulation is described. In this method, Phosphomolybdotugstic mixed acid of the folin ciocalteu reagent (FCR) is reduced by the dasatinib in presence of sodium hydroxide to give a blue colour product, having absorption maxima at 745 nm. The method is simple, rapid, sensitive and easy to apply in routine usage and do not require any costly instrumentation.

MATERIALS AND METHODS

Apparatus

Absorbance measurements were made on Shimadzu UV-1700 UV visible spectrophotometer with 10 mm quartz cells. Whatman filter paper no.42 was used to filter the solution.

Chemicals and reagents

All chemicals were of analytical reagent grade and solutions were prepared with doubled distilled water. Dasatinib (DTB) was purchased from M/s Hwasun Biotechnology Co. Ltd, Shanghai, China. Folin ciocalteu reagent (FCR) and sodium hydroxide (NaOH) was purchased from E Merk (India), HCl purchased from E Merk (India), Allied chemicals (India), Suvidhinath Laboratories (India).

Optimization of parameters

Concentration and volume of reagent

DTB was found to yield blue colored product with FCR and NaOH and having absorbance maxima at 745 nm. Therefore, investigations were carried out to establish the most favorable conditions for the formation of this colored product.

The influence of the concentration as well as volume of reagent on the reaction has been studied. Different concentrations and different volumes were tried for all the reagents.

Temperature and time

The stability of developed chromogen was assessed under two different temperature conditions, i.e., at room temperature and at 40^0 C. For color stability, aliquots of concentration 10, 20, 40, 60, and 80 µg/ ml were prepared and complex was made, as per procedure given in previous section. The samples were kept in transparent and amber colored glass vials under room temperature and elevated (40 ± 2 ^{0}C) temperature conditions, in controlled oven for varying period of time. The samples were analyzed initially and then periodically at 10, 20, 40, 60, and 80 min for absorbance determination.

Preparation of standard solutions and calibration curve

Standard stock solution of DTB

A stock solution was prepared by dissolving 50 mg of DTB in required quantity of 0.1 M HCL and diluting to 50 ml with same solvent. From the above solution 2.5 ml was again diluted to 25 ml with purified water to get 100 µg/ml solution of DTB.

Standard solution of FCR

A standard solution of FCR was prepared by diluting the reagent with double distilled water to get a concentration of 1 N.

Standard solution of NaOH

A standard solution of sodium hydroxide[7] was prepared by dissolving 4 gm of reagent in sufficient quantity of double distilled water and finally diluting to 100 ml with double distilled water.

Procedure for calibration curve

Suitable aliquots of the drug solution (1 to 8 ml) were taken in 10 ml volumetric flasks. To each flask was added 0.8 ml of standard FCR solution, 6.0 ml of purified water, and all the flasks were shaken well for at least 2 to 5 min., followed by addition of 1.0 ml of standard NaOH solution. Finally volume was made up to the mark with purified water to prepare a series of standard solutions containing 10 to 80 µg/ ml DTB. The absorbance of blue color chromogen was measured at 745 nm against reagent blank within one hour and the calibration curve was plotted.

Method validation[8]

Accuracy of the methods was determined by recovery studies in the tablet formulation of DTB. Recovery studies were carried out by

addition of known quantities of standard drug solution to pre-analyzed sample. Also, the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The percent coefficient of variance (% CV) was calculated at each concentration level.

The reproducibility was confirmed by repeating the methods, taking HCL from three different manufacturers and by three different analysts, and the percent relative standard deviation (% RSD) was calculated. The values of method validation are given in (table 3). The proposed method obeys beer's law in the concentration range of 10-80 µg/ml. In the method, the correlation coefficient was found to be 0.9991, the slope was 0.0111 and the intercept was 0.0098. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by repeating the blank measurements twelve times at 745 nm. The values were found to be 0.156 µg/ ml and 0.489 µg/ ml respectively (table 4).

RESULTS AND DISCUSSION

The main object of the study was to develop an accurate, precise, sensitive and reproducible method for determination of dasatinib. For this, colored complex of dasatinib was formed with the help of FCR in presence of NaOH, and its application in analytical detection was explored. This formed complex was blue in color and showed wavelength of maximum absorbance (λmax) at 745 nm. The stability of color as well as the developed complex is a prerequisite towards such motif. Hence, the stability of this complex was assessed under different conditions of temperature, time and concentration of reagents.

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. By keeping one constant at a time and the optimum concentration of FCR was 1 N and of NaOH was 1 N. Similarly optimum volume of FCR and NaOH was found to be 0.8 ml and 1 ml respectively. Whereas it was found that the complex was stable at room temperature, and showed no change in absorbance value throughout the study (figure 1).

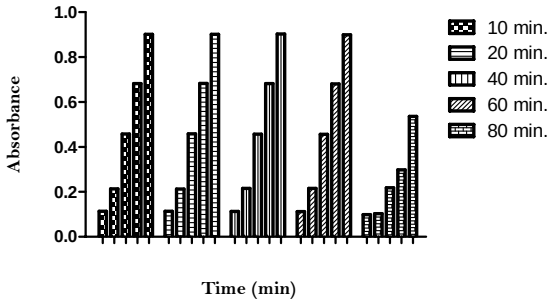

Fig. 1: Stability of color at room temperature

The solution was also found to be retaining its stability for 1 h (figure 2); so it is recommended that the reading should be taken within the specified time range.

On other side, it should be noted that the color of complex starts fading when exposed to higher temperature 40 ± 2° C (figure 3). This suggests that the exposure of these colored solutions to high temperature should be avoided during the analysis.

The proposed method is simple, rapid, precise and do not suffer from any interference due to common excipients of tablet. Beer's law is obeyed in the concentration range of 10-80 µg/ml. The method was validated in terms of accuracy, precision and reproducibility; the results are recorded in table 1 and 3. The accuracy of the method was proved by analyzing the synthetic mixture and tablet formulations. Values greater than 99.0% indicate that the proposed method is accurate for the analysis of drug (table 1).

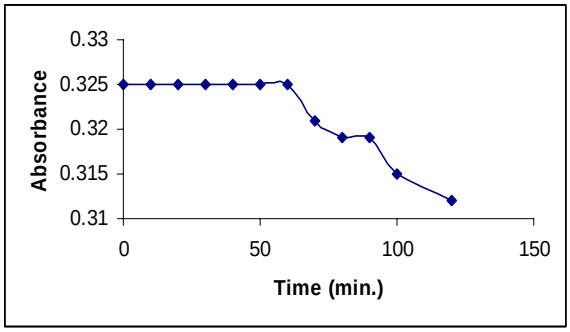

Fig. 2: Duration of stability of color

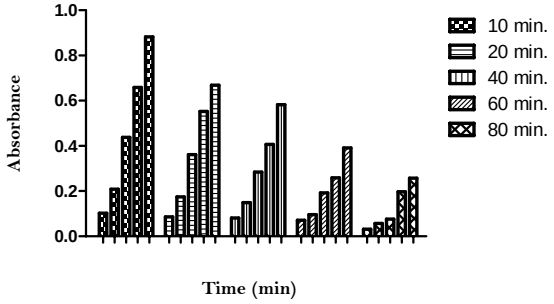

Fig. 3: Stability of color at 40° C temperature

Table 1: Analysis of synthetic mixture and tablet dosage form

Dosage form	Label claim (mg/tablet)	Amount found (mg/tablet)	% Label claim*
S.M.	10	9.95	99.56 ± 0.1232
Tablet-1	20	20.04	100.20 ± 0.6952
Tablet-1	50	49.99	99.98 ± 0.2125

*average of six determination

The recovery studies were carried out by adding known amount of standard solution of DTB to preanalysed drug solutions. The resulting solutions were then analysed by the proposed method. The results of recovery studies were found to be satisfactory and the results are presented in table 2.

The precision of the proposed method was checked in terms of inter-day and intra-day, where method was repeated on three different days and also repeated for three different time periods in the same day. The results given in table 3 showing % CV of less than 1% at each level clearly indicate that the proposed method is precise enough for the analysis of drug. The reproducibility of the method was checked by getting the proposed method performed by three different analysts and by taking solvent from three different manufacturers. The values of % RSD less than 1% (table 3) indicate that the proposed method is reproducible for the analysis of DTB.

Table 2: Recovery studies

Conc. of formulation (µg/ml)	Std. spiked (µg/ml)	Total conc. taken (µg/ml)	Total conc. found (µg/ml)	% recovery*
10	2	12	11.84	99.05 ± 0.42
10	3	13	12.92	99.46 ± 0.33
10	4	14	14.01	100.12 ± 0.42
30	2	32	32.10	100.32 ± 0.52
30	3	33	32.92	99.76 ± 0.34
30	4	34	33.75	99.28 ± 0.36

*average of six determination

Table 3: Precision data and reproducibility data

Interday precision (%CV) *	Intraday precision (%CV) *	Reproducibility (%RSD) *
0.698	0.782	0.526

*average of six determination

The optical characteristics, such as beer's law limit, molar absorptivity, sandell's sensitivity[9], are recorded in table 4. The regression analysis using the method of last sequence was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations. The results are summarized in table 4. Rigorous analysis of the results shows that the presence of excipients in tablet formulation did not interfere with the final determination of the active component, DTB. This reveals the potential utility of this developed method for the routine analysis of DTB in bulk and in pharmaceutical preparations.

Table 4: Summarized optical characteristics and other parameters

Parameters	Result
λ max (nm)	745
Color of chromogen	Blue
Beer's law limit (µg/ml)	10 to 80
Molar extinction (l/mol.cm)	$1.026 X10^4$
Sandell's sensitivity (µg/cm^2 per 0.001 absorbance unit)	0.01996
Regression equation (Y= mX + c)	0.0111x + 0.0098
Slope	0.0111
Intercept	0.0098
Limit of detection (µg/ml)	0.156
Limit of quantification (µg/ml)	0.489
Coefficient of determination	0.9991
% RSD	< 1%
Accuracy	> 99%

REFERENCES

1. Luo FR, Yang Z, Camuso A, Smykla R, McGlinchey K, Fager K et al. Dasatinib (BMS-354825) pharmacokinetics and pharmacodynamic biomarkers in animal models predict optimal clinical exposure. Clin Cancer Res 2006; 12:7180–7186.
2. Lee FY, Lombardo L, Camuso A, Castaneda S, Fager K, Flefleh C et al. Potently inhibits multiple selected oncogenic tyrosine kinases and possesses broad-spectrum antitumor activities in vitro and in vivo. In: AACR 96th annual meeting 2005.
3. Lombardo LJ, Lee FY, Chen P et al Discovery of N-(2- chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino) thiazole-5-carboxamide (BMS-354825). A dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem 2004; 47:6658– 6661.
4. Tokarski JS, Newitt JA, Chang CYJ et al. The structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. Cancer Res 2006; 66:5790–5797.
5. Mhaske DV, Dhaneshwar SR. Stability indicating HPTLC and LC determination of dasatinib in pharmaceutical dosage form. Chromatographia 2007; 66: 1-2.
6. Gowri Sankar D, Rajeswari A, Nagesh A, Vamsi Krishna M. UV-spectrophotometric determination of dasatinib in pharmaceutical dosage forms. Asian J.Chem 2009; 21: 5777-5779.
7. Indian Pharmacopoeia, 4th Ed. New Delhi: The controller of publications, government of India, New Delhi 1996.
8. Ahuja S, Scypinski S. Handbook of modern pharmaceutical analysis. Academic Press, USA 2001; p- 430.
9. Sandell EB. Colorimetric determination of traces of metals, 3rd Ed. Interscience Publication; New York: USA 1965, p-85.