DEVELOPMENT OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DASATINIB IN PHARMACEUTICAL FORMULATION AND BIOLOGICAL SAMPLES

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ABSTRACT

A simple, economical, accurate, precise and reproducible UV-Visible spectrophotometric method for the routine estimation of dasatinib has been developed. The method is based on the formation of a bluish green colored complex by dasatinib in presence of MBTH reagent. The developed colored complex showed λmax at 630 nm. Beer's law in the concentration range of 10 to 60 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 pharmaceutical formulation and biological samples, with an average recovery between 99 to 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.

Keywords: Dasatinib, UV-Visible spectrophotometric, Method development, pharmaceutical formulation and biological samples.

INTRODUCTION

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 large quantities or are more active in cancer cells. Growth and survival of cancer cells are reduced by reducing the activity of these proteins. The chemical name for dasatinib is N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-iazolecarboxamide monohydrate. 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, soluble in acetonitrile and slightly soluble in ethanol and methanol. dasatinib is an inhibitor of multiple tyrosine kinases [1-2]. High-performance liquid chromatographic method is used for the determination of dasatinib in rabbit plasma using fluorescence detection and its application to a pharmacokinetic study [3] .Furthermore, dasatinib inhabits the viability of both non-small cell lung cancer, head and neck squamous cell cancer [4]. Literature survey revealed that few analytical methods such as HPLC [5-6], LC-MS [7-8] and UPLC [9] 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 the antileukemia drugs like 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 response 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 are determined its main metabolite. (CGP-74588) in human plasma by ultra-violet high performance liquid chromatography has been reported [17].HPLC–MS method for the simultaneous quantification of the antileukemia drugs Imatinib, dasatinib and Nilotinib in human peripheral blood mononuclear cell is also reported [20]. A validated LC–MS/MS assay for the simultaneous determination of the anti-leukemic agent dasatinib and two pharmacologically active metabolites inhuman plasma are reported in the literature [24].Liquid chromatographic–mass spectrometric method for the determination of cellular levels of the tyrosinekinase inhibitors lapatinib and dasatinib are also reported in the literature [26]. Simultaneous

measurement of Imatinib, nilotinib and dasatinib in dried blood sample using by ultra high performance liquid chromatography tandem mass spectrometryis reported [30].Simultaneous analysis of anticancer agent's bortezomib, Imatinib, Nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS is are also reported in the literature [31].

However, there is no reported UV- Visible Spectrophotometric method for the analysis of dasatinib in its pharmaceutical formulation and biological samples. Validated UV- Visible Spectrophotometric method for the quantitative determination of dasatinib, the functional group used for color development of dasatinib is amine group. The results obtained in this method were based on the Oxidative coupling reaction with MBTH/ Ferric chloride.

EXPERIMENTAL

Preparation of standard stock solution

Accurately weighed 100 mg of dasatinib was dissolved in 40 ml of methanol in a 100 ml volumetric flask and it was made up to the mark with methanol. i.e. 1000 µg ml^{-1} (Stock solution A). From the above (stock solution A). 10 ml of aliquots was pipetted out into 100 ml volumetric flask and it was made up to the mark with methanol to obtain the final concentration of 100 µg ml^{-1} (Stock solution B).

Preparation of Calibration curve

Fresh aliquots of dasatinib ranging from 1 to 6ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 10 to 60 µg ml^{-1}. To each flask 1ml of (0.5%) MBTH solution was added followed by 1ml of (0.7%) ferric chloride solution and resulting solution was heated for 15 min and finally 1ml (0.5N) hydrochloric acid was added. The solutions were cooled at room temperature and made up to mark with methanol. The absorbance of bluish green colored chromogen was measured at 630 nm against the reagent blank. The color species was stable for 32 hrs. 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 solvent methanol was evaporated. The residue obtained was dissolved in a 100 ml volumetric flask to get the stock solution A. 10 ml of aliquots were pipetted out into a 100 ml volumetric flask and the volume was finally made up to the mark with methanol. This was help to get final concentration of 100µg ml^{-1} (Stock solution B). Subsequent dilutions of this solution were made with methanol to get concentration of 10 to 60 µg ml^{-1}. These were analyzed at the selected wavelength, 630 nm and the results were statistically validated.

Procedure for Blood sample

Blood sample collected were 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 aforesaid solutions ranging from 0.5-3 ml (5-30 µg ml^{-1}) were transferred in to 10 ml Volumetric flask and to the each flask 1ml of (0.5%) MBTH solution was added followed by 1ml of (0.7%) Ferric chloride solution and made up to the mark with methanol. Then the resulting solution was heated and finally 1ml (0.5N) hydrochloric acid solution was added. The solutions were cooled to room temperature and made up to the mark finally with methanol the absorbance of blush green colored chromogen was measured at 630 nm against the reagent blank. The color species were stable for 32 hr. The amount of dasatinib present in the sample solution was computed from its calibration curve.

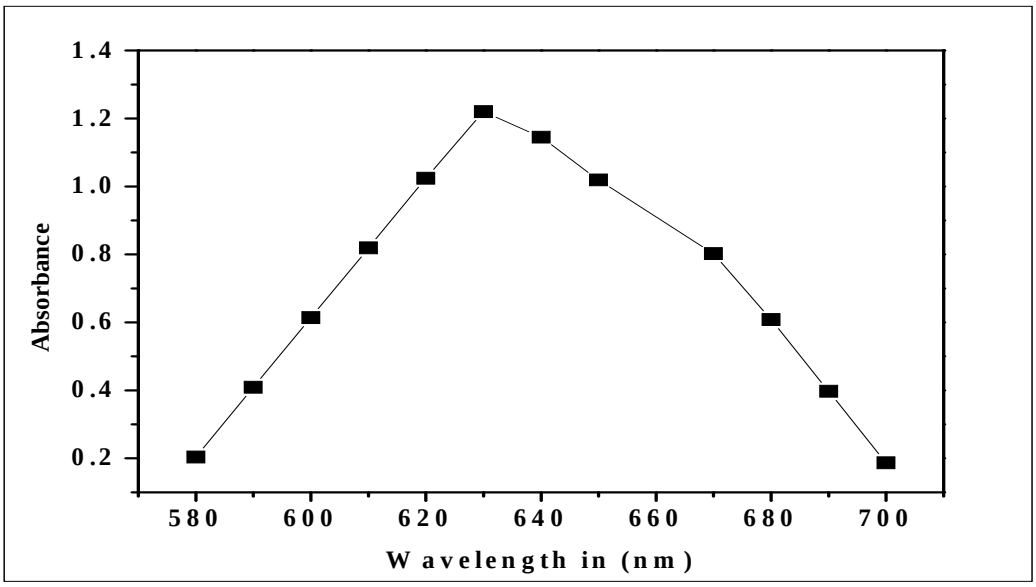

Fig-1: Absorption spectrum of Dasatinib with MBTH /$FeCl_3$

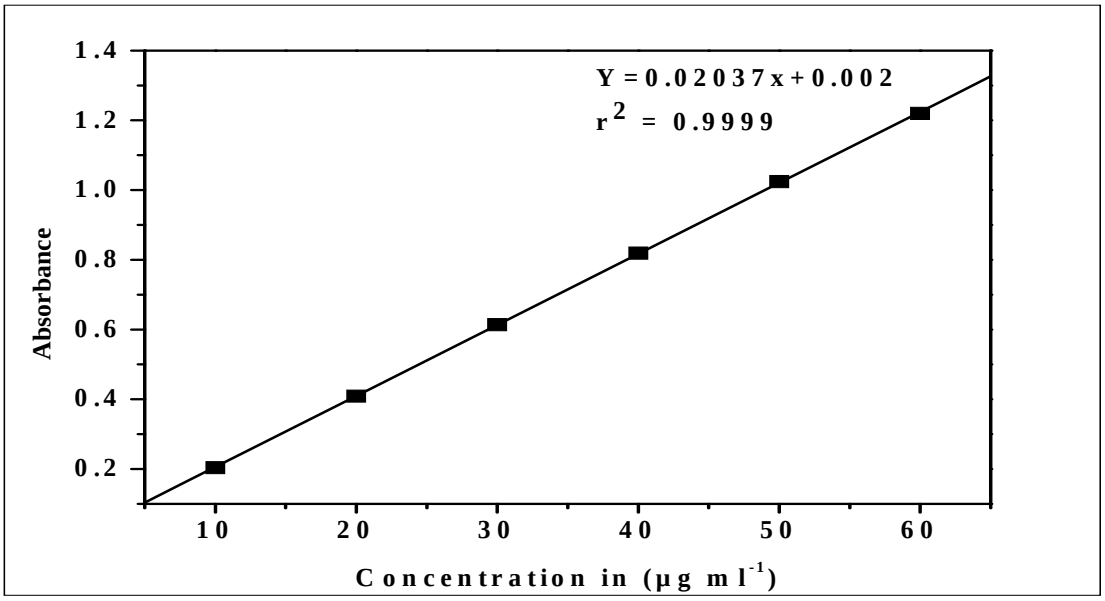

Fig-2: Beer's law plot of Dasatinib with MBTH/$FeCl_3$

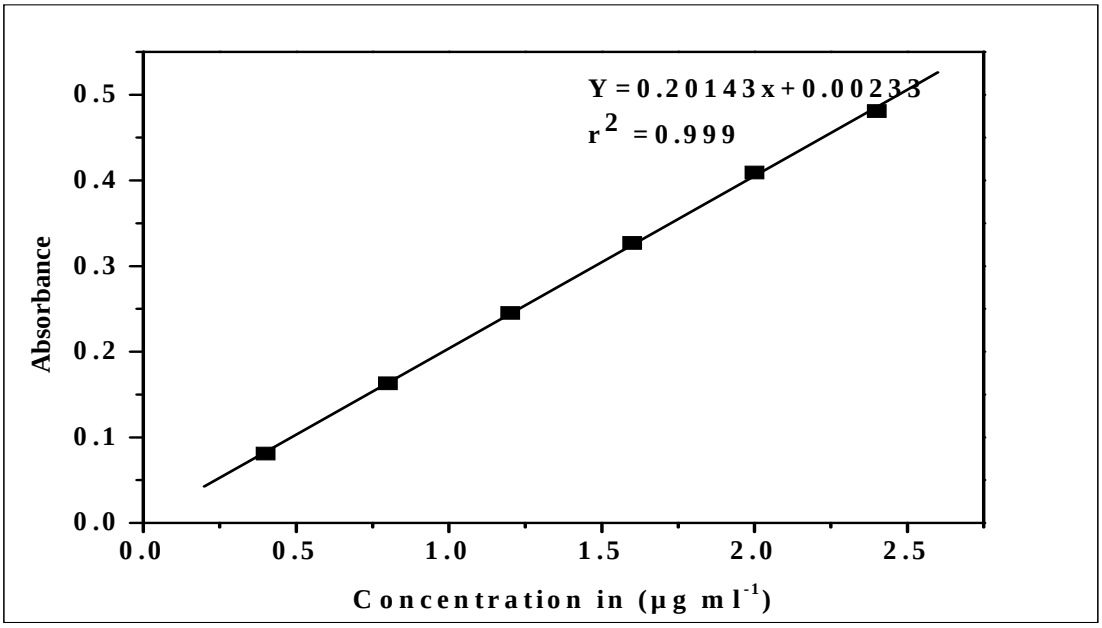

Fig-3: Beer's law plot for MBTH in Blood sample

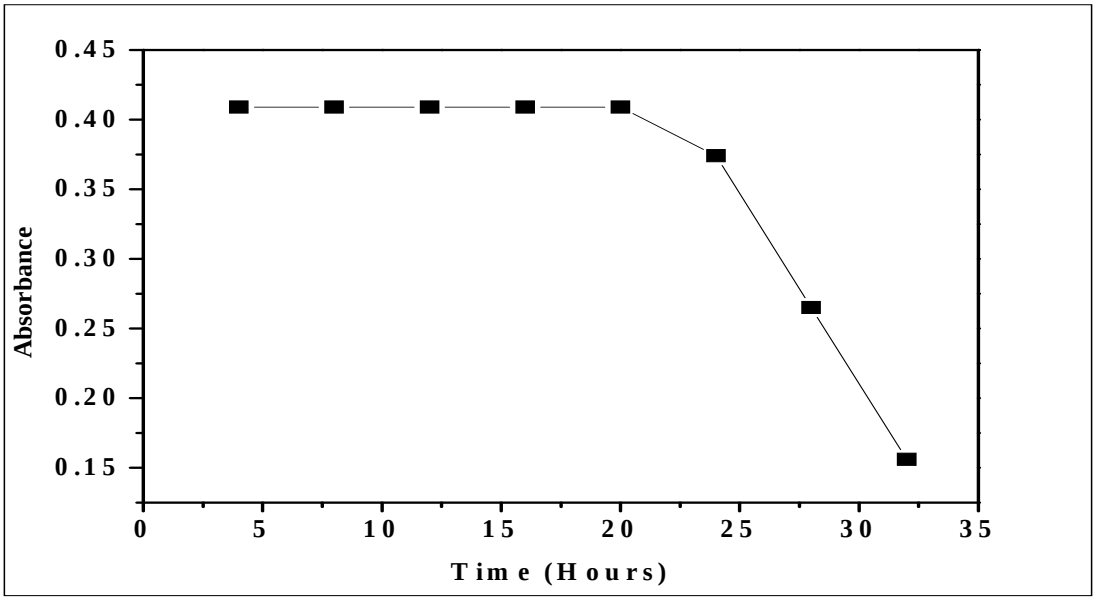

Fig-4: Color stability data for MBTH Method

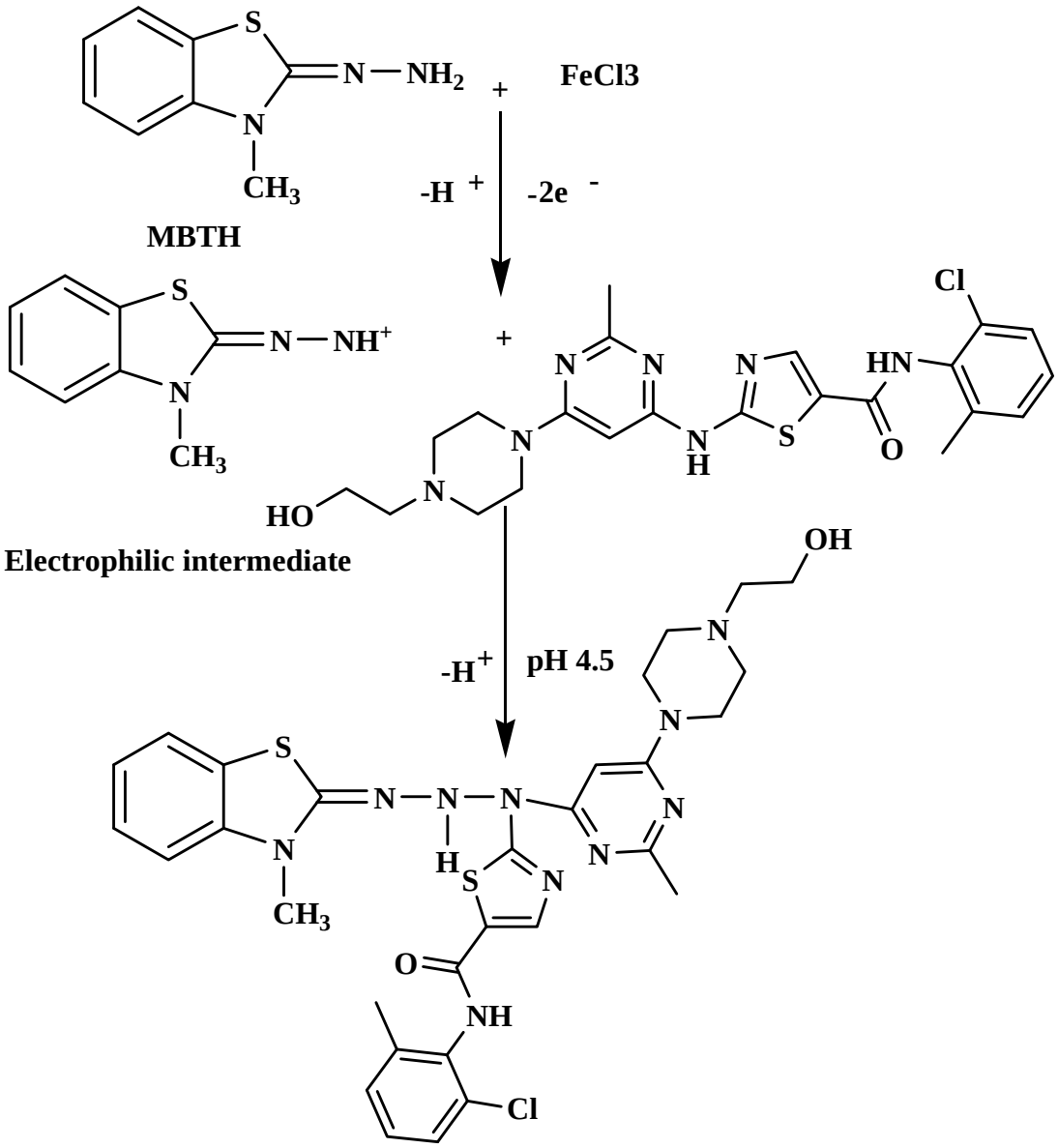

Fig-5: A Schematic reaction Mechanism of Dasatinib with MBTH

Table-1: Optical characteristics and precision by MBTH

Parameter	Visible method
Color	Bluish Green
Absorption maxima (nm)	630
Beer's law limits (µg ml^{-1})	10-60
Molar absorptivity (l $mol^{-1}cm^{-1}$)	$0.02033x10^4$
Sandell's Sensitivity (µg cm^{-2})	0.81967
Regression equation (Y*)	Y=bc+a
Slope (b)	0.02037
Intercept(a)	0.002
Standard deviation(SD)	0.00311
Correlation coefficient (r^2)	0.9999
%RSD (Relative Standard deviation)	0.54742
Limits of detection (LOD)(µg ml^{-1})	0.45802
Limits of quantification (LOQ) (µg ml^{-1})	1.52675

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	249.98 T=0.002967 F=3.80671	249.20	99.98
NILOTINIB	250	249.95 T=0.002966 F=3.83576	248.57	99.95

- 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
249.95 249.64 249.34	200 200 200	449.91 449.35 448.81	99.91 99.35 98.81
249.90 249.85 249.79	250 250 250	499.8 499.7 499.58	99.8 99.7 99.58
249.89 249.79 249.60	300 300 300	549.75 549.53 549.31	99.75 99.53 99.31

Table-4: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
449.35	0.55003	0.12240
499.69	0.11015	0.02204
549.53	0.22	0.04003

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

Table-5: Repeatability data for DSB at 630 nm MBTH

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%)RSD
10	0.204	0.202	0.201	0.2023	0.00153	0.75630
20	0.409	0.406	0.408	0.4076	0.00153	0.37536
30	0.614	0.612	0.610	0.612	0.002	0.32679
40	0.819	0.816	0.818	0.8176	0.00153	0.18713
50	1.024	1.020	1.021	1.0216	0.00208	0.20360
60	1.220	1.221	1.209	1.2166	0.00666	0.54742

Average of six determinations.

Table-6: Color stability data for MBTH Method

Conc. in µg ml^{-1}	Time in Hours							
20	4	8	12	16	20	24	28	32
	0.409	0.409	0.409	0.409	0.409	0.374	0.265	0.156

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=0.002969 F=3.6013	1.87	99.95
NILOTINIB	2	1.90 T=0.002968 F=3.5951	1.89	99.90

- Tand F values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits t=0.00196 and F=2.5961.

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	1.90	2	3.8	99.8

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

Table-9: Repeatability data for Dasatinib at 630nm

Concentration in µg ml^{-1}	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
04	0.081	0.080	0.081	0.0806	0.00058	0.71960
08	0.163	0.162	0.163	0.1626	0.0005	0.30750
12	0.245	0.245	0.244	0.2446	0.00058	0.23712
16	0.327	0.326	0.327	0.3266	0.00058	0.17758
20	0.409	0.408	0.407	0.408	0.001	0.24509
24	0.481	0.480	0.481	0.4806	0.0005	0.10403

Average of six determinations

Method

The results obtained in this method were based on oxidation followed by coupling reaction of dasatinib with MBTH, Ferric chloride and HCl these results in the formation of a blush green colored chromogen that exhibited maximum absorption at 630 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of dasatinib with MBTH reagent was shown in (fig-5). The effect of various parameters such as concentration and volume of MBTH and strength of acid was studied by means of control experiments varying one parameter at a time.

Optical Characteristics

The reference method to follows beer's law was studied 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. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. Beer's law limits, Molar absorptivity & sandells 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. The beers law plots of the system are illustrated graphically (fig-2&3) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity Sandells 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 three proposed spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of a fixed amount of dasatinib in 10 µg ml^{-} in a final solution. The percent relative standard deviations were calculated for the proposed methods and presented in table –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 the comparison showed that those proposed methods do not differ significantly from the reported methods and they are presented in table-1. The proposed methods also applied for biological Samples (Blood) and good recoveries are obtained which are recorded in table-7.

Accuracy

Recovery studies were carried by applying the Standard addition method to drug samples present in formulations for the known amount of dasatinib. By applying the same method to biological sample (Blood) to which known amount of dasatinib correspond to 2 mg formulations taken by the patient. By the follow of Standard addition method 2 mg of label claim was added. And after 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 a 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 these presented in Table –3. The results obtained 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 an 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 six set of solutions having formulations were spiked in a pre weighed quantity of drug and then absorbance was measured and calculations were done to determine the quantity of the drug.

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 measured six times and standard deviation was calculated and the results are presented in table -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 that no significant change occur in assay values indicating stability of drug in the solvent used during analysis. The results are given 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 were present in excess.

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.

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