NEW VISIBLE SPECTROPHOTOMETRIC ASSAY OF IMATINIB (β-FORM) IN PURE AND FORMULATIONS

BV Sreenivasulu[1] and C Rambabu[+]

1. Department of Biochemistry, Acharya Nagarjuna University, Guntur, AP, India

[+] Department of chemistry, Acharya Nagarjuna University, Guntur, AP, India

ABSTRACT

Two simple, sensitive and rapid spectrophotometric method for the determination of imatinib (β-form) in pure as well as in pharmaceuticals were described. The proposed methods are based on the formation of ion – association complexes of the drug with BCG and BTB, which were measured at absorption maximum of 414nm and 416nm respectively. Beer's law was obeyed in the concentration ranges 10.0 to 50μg/ml for BCG and BTB methods, respectively. Other statistical analyses such as Student's t test and F test values are studied for both the proposed methods and the results were with that of the reported spectrophotometric methods. Basing on the results the proposed methods can be successfully applied for the assay of imatinib(β-form) in various forms of pharmaceuticals.

INTRODUCTION

Imatinib[1] is 4-4[(4-methyl-1- piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl] – benzamide mono methane sulfonate used to treat leukemia and gastrointestinal tumors**.**

Literature Survey revealed that the drug has been estimated by Liquid chromatography [2-9] and Spectrophotometry [10,11] methods in biological fluids like human plasma and rat plasma and HPLC method in pharmaceutical formulations has been reported so far. But no visible spectroscopic method was reported for the estimation in pure and pharmaceutical dosage forms. The present paper describes two new highly sensitive, rapid, simple, economical visible spectrophotometric assay for imatinib (β-form) in pure and in formulations by exploiting its analytically useful functional groups and its ability to form ion-pair complex with two acidic dyes BCG and BTB.

MATERIALS AND METHODS

APPARATUS: A double beam UV-Visible Double beam spectrophotometer (Elico SL-160 A) with matched 1.0cm quartz cuvettes was used for all absorbance measurements. A digital pH meter Model Elico L1 120 was used for pH measurements.

CHEMICALS AND MATERIALS: Imatinib (β-form) (pure drug) used was obtained from Micro Labs Ltd as gift sample with 99.9% w/w assay value. All the Solvents and other chemicals used were of analytical grade and double distilled water was used throughout the investigation. Solutions of BCG 0.2% (w/v) BTB 0.2% (w/v) were prepared separately in double distilled water freshly. Phosphate buffer of pH-3.2 was prepared by dissolving 900ml of a 4.0g/l solution of sodium dihydrogen phosphate and 100ml of a 2.5g/l solution of phosphoric acid in 1000ml volumetric flask. Adjust the pH3.2 if necessary.

PREPARATION OF STANDARD STOCK SOLUTION: Standard drug solution of imatinib (β-form) (stock) was prepared by dissolving 10mg in 10ml methanol (1.0mg/ml). The working standard solution of imatinib (β-form) (200μg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

2

ASSAY PROCEDURE FOR TABLETS: Ten tablets were weighed and pulverized to a fine powder. The amount of tablet powder equivalent to 10mg of imatinib (β-form) was weighed accurately and transferred 100ml volumetric flask. Then 10ml methanol was added and kept for 15min with frequent shaking and volume was made up to mark with methanol. The solution was then filtered through Whatmann filter paper #45. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation and was proceeded as stated in "recommended procedure"

RESULTS AND DICUSSION

i) METHOD DEVELOPMENT: It involves the optimization studies of each proposed method that directly influences the color development [optimal conditions]. The optimum conditions for each proposed method were established by varying one variable and observing its effect on the absorbance of the colored products. It involves the careful study of the effect of various parameters which include effect of reagent concentration, order of addition, time, temperature and choice of solvent on color development.

BCG & BTB: The optimum conditions necessary for the formation of the colored products with maximum stability and sensitivity for imatinib(β-form), the author performed control experiments by measuring absorbance at 414 & 416nm of a series of solutions, varying one and fixing the other parameters in each case such as type and volume of acid, concentration of dye, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature.

For BCG: Into a series of 125ml separating flasks containing 200µgml of the drug solution, keeping one reagent amount as constant, the concentration of the other reagent was varied and the mixture was diluted up to the mark with water. After 10 min absorbance of each solution was measured at 414nm. It was found that a 0.2% solution of BCG in the range 3.0 to 4.0 mL and pH 3.2 buffer in the range 4.5 to 5.5 ml were necessary to achieve maximum color intensity. Therefore, 2.0 ml of BCG was recommended for all measurements. The effect of time on maximum absorbance was also tested by measuring the absorbance of the colored solution at regular intervals and it was found that solutions show maximum absorbance after 10 min and was stable for 24hrs respectively.

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

3

For BTB: Same conditions as described for were followed for this method.

ii) ASSAY PROCEDURES:

With BCG: Aliquots (0.5-2.5ml) of standard solution of imatinib(β-form) (200μg/mL) were transferred to 125ml stoppered separating flasks. The final volumes in each separating flasks was adjusted to 5.0ml by adding phosphate buffer solution (pH 3.2). To the above flasks then add 2.0ml of BCG solution and were shaken for 5 min. Later 10ml of chloroform were added to the tubes and the mixtures were shaken for 2 min and allowed to stand for 5 min for separation of the chloroform layer. The absorbance of the organic phase was measured after 10min in 1.0cm quartz cells at 414nm against blank solution, which was prepared similarly calibration curve, was plotted using absorbance-values versus concentration (**Fig.2.04**).

With BTB: Same procedure described in the above method(BTB) was followed for varying aliquots of standard solution of imatinib (β-form) (200μg/mL) and the absorbance of imatinib(β-form)-BTB complex was measured at 416nm against the reagent blank as reference respectively. The concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using the absorbance concentration data (**Fig.2.05**).

iii) METHOD VALIDATION:

a) SPECTRAL CHARACTERISTICS: The absorption spectra were scanned on a spectrophotometer in the wavelength region of 340 to 500nm against similar reagent blank or distilled water and the results were graphically represented in **Fig.2** for **BCG,** and **Fig.4** for **BTB** respectively. The absorption curves of the colored species in each method showed an characteristic absorption maxima whereas the blank in each method exhibited no absorption in this region.

b) OPTICAL CHARACTERISTICS: The Beer's law plots and Ringbom plots (**Fig.3&5**) of the developed methods were recorded graphically for imatinib(β-form). Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range, LOD and LOQ for lipatinib in each method were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient and their values were tabulated in **Table.1**.

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

4

c) PRECISION: The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of imatinib(β-form) in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (**Table.1**).

d) RECOVERY STUDIES (ACCURACY): The recovery studies were conducted by analyzing each pharmaceutical formulation in the instance for the active ingredient by the proposed methods. Known amount of pure drug was added to each previously analyzed formulation and the total amount of the drug was once again determined by all proposed methods after bringing the active ingredient concentration within the Beer's law limits. The results are reported in **Table.2**.

e) ANALYSIS OF FORMULATIONS: Commercial formulations (tablets) containing imatinib (β-form) were successfully analyzed by the proposed methods using the reported method. The precision and accuracy of the developed methods was further compared statistically using Student's t test and variance ratio F test. At a 95% confidence level, the calculated t-values and F-values do not exceed the tabulated values and the results of percent recoveries by the proposed methods were summarized in **Table.3** revealing that the data are consistent with the label claim.

f) INTERFERENCE STUDIES: The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of imatinib(β-form)under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

g) NATURE OF THE COLORED SPECIES: An attempt has been made by the author to indicate the nature of colored species in each of the proposed methods for imatinib(β-form) is based on analogy [reactive functional moiety (tertiary nitrogen group) in drug, reagents nature.

For BCG and BTB: The proposed procedures are based on the reaction between imatinib(β-form) and BCG & BTB resulting in the formation of a yellow ion-pair complex which is extracted into chloroform and measured spectrophotometrically. The formation of the complex is shown in the reaction scheme (**Fig.6**) given below.

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

5

CONCLUSION

The proposed methods developed by the author made use of simple reagents (Acidic dyes), which most of the ordinary analytical laboratories can afford. The present methods involve the formation of highly stable colored species which makes it easier for the determination of imatinib(β-form) in pure and in pharmaceuticals at the given optimum conditions. Further, the results of statistical parameters and the recovery studies clearly indicated the reproducibility and high accuracy of the proposed methods. Therefore, it was concluded that the proposed methods are suitable and valid for application in assaying of imatinib(β-form) related drugs in laboratories lacking liquid chromatographic instruments.

ACKNOWLEDGEMENT

REFERENCES

1. Martindale: The complete drug reference. 36th edition, Pharmaceutical press, Lambeth High Street, London. 2009; 773-774.

2. Widmer et al. Determination of imatinib in human plasma by solid-phase extraction–liquid Chromatography–ultraviolet absorbance detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2004; 803(2): 285-92.

3. Thirumurthy et al. Development and validation of a simple liquid chromatographic method with ultraviolet detection for the determination of Imatinib in biological samples. J. of Chromatography B. 2004; 804, 2(25):431-434.

4. Vivekanand et al. A validated LC method for imatinib mesylate Journal of pharmaceutical andbiomedical analysis, 200; 333(5): 879-889.

5. Ivanovic, D., Medenica, M., Jancic B. and Malenovic, A., Reversed-phase liquid chromatography analysis of imatinib mesylate and impurity product in Glive capsules. J. chromatography B. 2004; 800 (1-2): 253-258.

6. Roos, LO., Jos, HB., Jan HM.,Olaf van, T. Determination of imatinib mesylate and its main metabolite (CGP74588) in human plasma and murine specimens by ion-pairing reversed phase high-performance liquid chromatography. Biomedical chromatography 2007; 21(7):747-754.

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

6

7. Solassol, F., Bressolle, L., Philibert, V., Charasson, C., Astre, F.Liquid Chromatography- Electrospray Mass Spectrometry Determination of Imatinib and Its Main Metabolite, *N*-Desmethyl- Imatinib in Human Plasma. J. liquid chromatography and related technologies. 2006;29 (20): 2957-2974.

8. Rosasco et al. Validation of an HPLC Method for the Determination of Imatinib Mesylate in Pharmaceutical Dosage forms. J. liquid chromatography and related technologies, 2005;28 (20): 3283-3292.

9. Satyanarayana et al. Development and Validation of New Reversed Phase High Performance Liquid Chromatography Method for the Estimation of Imatinib in Bulk and Pharmaceutical Dosage Forms. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2010; Vol. 1 (1): 6-9.

10. Bende, G., Kollipara, S., Sekar, V. and Saha, R. UV-spectrophotometric determination of Imatinib mesylate and its application in solubility studies.Die pharmazie, 2008; 63(9): 641-645.

11. Ms. Patil Smita J., Dr. Doijad Rajendra C., Ms. Dhumal Priya P. Development of Uv-Spectrophotometric Method for the determination of Imatinib Mesylate (Itm) in Bulk And Formulation, Asian J Pharm Clin Res, Vol 6, Issue 3, 2013, 54-57.
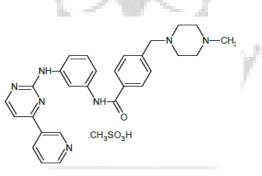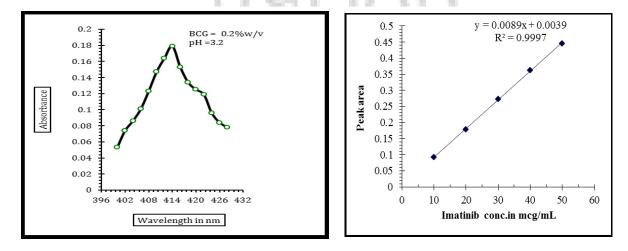
Figure 1: Structure of Imatinib

Fig.2&3: Absorption spectra and Beer's Law plot of IMB with BCG

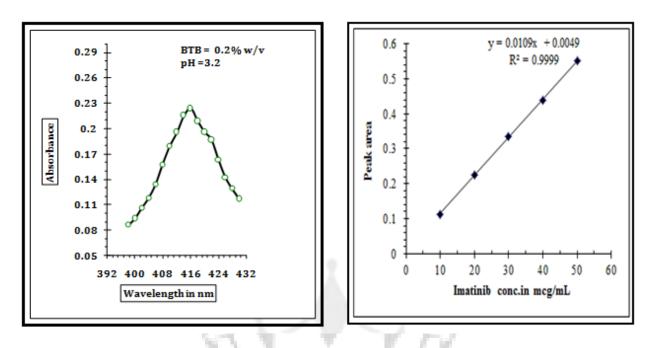*Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.*

7

Fig.4&5: Absorption spectra and Beer's Law plot of IMB with BTB

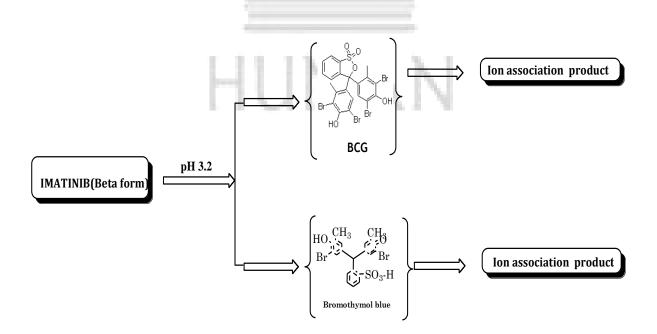
Fig.6: Reaction scheme of IMB with BCG and BTB

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

8

TABLE.1 RESULTS OF METHOD VALIDATION OF THE PROPOSED METHODS FOR IMATINIB (β-FORM)

Parameter	BCG	BTB
λ_{max} (nm)	414	416
Beer's law limits (µg/ml)	10 - 50	10 - 50
Molar absorptivity (1 mol^{-1}.cm^{-1})	2.998×10^3	2.971×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 A.U)	6.486×10^{-2}	6.238×10^{-2}
Regression equation (Y=a+bc);Slope (b)	0.0089	0.0109
Intercept (a)	0.0039	0.0049
Correlation coefficient (r)	0.9997	0.9999
Relative standard deviation (%)*	1.19	1.12
% Range of error (confidence limits)		
0.05 level	1.012	0.937
0.01 level	1.437	1.38
LOD	0.0097	0.0094
LOQ	0.059	0.0181

* Average of six determinations considered.

TABLE.2. THE RESULTS OF (ACCURACY STUDIES) OF IMATINIB (β-FORM) BY THE PROPOSED VISIBLE SPECTROPHOTOMETRIC METHODS

Proposed methods	IMB in tablet µg.mL-1	Pure IMB added µg.mL-1	Total found µg.mL-1	Pure IMB recovered %±SD*
BCG	10.0	5.0	14.93	99.53
BTB	10.0	5.0	14.96	99.73

* Average of six determinations considered.

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

9

TABLE.3 ASSAY OF IMATINIB (β-FORM) IN PHARMACEUTICAL FORMULATIONS

	Proposed methods	BCG	BTB
Assay of Imatinib(β-form) in pharmaceutical Formulations**	**Amount found by the Reference Method[11]**	100.2± 0.23	
	Amount found	99.81± 0.16	99.84± 0.14
	F- test	2.44	3.19
	t-Test	0.845	0.62
	% Recovery	99.61	99.64

** Average $\pm$ standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

Citation: BV Sreenivasulu et al. Ijppr.Human, 2014; Vol. 2 (1): 1-10.

10