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Der Pharmacia Lettre, 2012, 4 (3):720-727
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ISSN 0975-5071
USA CODEN: DPLEB4

UV Visible spectrophotometric determination of bortezomib in its bulk and formulation dosage forms

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ABSTRACT

Literature survey has been revealed that there is no any visible spectrophotometric method has been reported for estimation Bortezomib in bulk form and in pharmaceutical formulation. Hence an attempt has been made to develop and validate a simple, economic, rapid, and accurate method. In present investigation three simple and sensitive extractive UV-Visible spectrophotometric methods have been developed and validated for the determination of bortezomib in formulation dosage form. The developed methods involve formation of non extractable Condensation complex of drug with Anthranilic acid ***(M-A),*** *2-chlorophenyl hydrazine* ***(M-C)*** *and Oxidative coupling complex with MBTH/Fe (III) (****M-B****) dyes in basic and acidic mediums. Non extractable complexes shows maximum absorption for M-A, M-B, and M-C at* ***485 nm****,* ***630 nm and 560 nm*** *respectively, Beer's law is obeyed within the limits* ***2.5-12.5 µg/mL, 5-25 µg /mL and 2.5-12.5 µg /mL*** *and molar absorptivity is found to be* $\mathbf{4.630x10^4}$ *,* $\mathbf{1.544x10^5}$ *and* $\mathbf{7.76x10^4}$ *for method-A, Method-B and Method-C respectively The colored chormophores were found to be stable for 40 min. 60 min and 30 min respectively.*

Key words: Bortezomib, UV-Visible method validation and Beer's law

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INTRODUCTION

Bortezomib is chemically [(1*R*)-3-methyl-1-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl) amino] propanoyl} amino) butyl] boronic acid **[Fig.1].** Its molecular formula is $C_{19}H_{25}BN_4O_4$ having molecular weight 387.237 g/mole. Bortezomib (originally codenamed PS-341) is the first therapeutic proteasome inhibitor to be tested in humans. It is approved in the U.S. for treating relapsed multiple myeloma and mantle cell lymphoma. In multiple myeloma, complete clinical responses have been obtained in patients with otherwise refractory or rapidly advancing disease. Bortezomib (**VALCADE®**, formerly PS-341) was approved for the treatment of patients with relapsed or refractory multiple myeloma in May 2003 by the US foods and drugs Administration and in April 2004 by the Committee for treatment of mantle cell lymphoma.

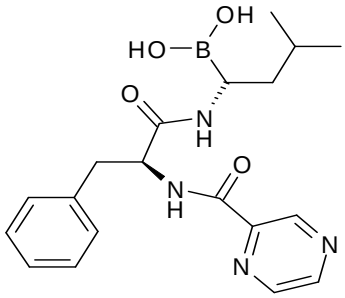


Figure-1: Chemical structure for Bortezomib (BMB)

As for as literature concern, few publications are available for bortezomib and are Vincent Chung, et. Al **[1]** have proposed a spectrophotometric method to determined the cell concentration by using KOVA Glasstic slides and **2.5 × 10^4** cells were plated in each well (Falcon 24-well plates), Du Zx, et al.,[**2]** have Identified Proteasome activity, intracellular glutathione (GSH) and ROS levels, as well as activities of GSH synthesis enzymes were measured using spectrophotometric methods, Fahy BN, et al [3] have developed a process to find the schedule-dependent molecular effects of the proteasome inhibitor bortezomib and gemcitabine in pancreatic cancer. Gerald H. Manorek, et al., [**4]** has reported in Clin. Cancer Res. January 15, 2009 15: 553-560., Enhanced Delivery of cisplatin to international Ovarian Carcinomas mediated by the effects of bortezomib on human copper transporter. Drug sensitivity was determined by clonogenic assay, platinum (Pt) levels were measured by inductively coupled plasma mass spectroscopy, copper (Cu) accumulation was quantified with ^{64}Cu, and the subcellular distribution of ATP7A was assessed by confocal digital microscopy and Cecilia Ceresa Elisa Giovannetti et al **[5]** has reported Modulation of gemcitabine Pharmacokinetics and Pharmacodynamics in non lung cell cancer and blood mononuclear cells by using LC MS.

J Scott Daniels et al has reported **[6]** as the characterization of bortezomib and metabolites observed in human plasma with the help of MDS sciex API 3000 triple quadruple LC MS using turbo ion spray interface set at 325^{0}C and Jeffrey S. Johnston et al has reported Pivotal studies of bortezomib by using LC MS in Human plasma in **[7]**.

In the current development the author proposed some UV-Visible methods to determine the bortezomib in its bulk and pharmaceutical injection dosage form are novel, economical, accurate, and stable and are very use full in pharmaceutical regular in house practices to reduce the cost.

COLOR CHEMISTRY

Chromogen chemistry of a molecule mostly depends on its functional groups and as well as on its chemical unsaturation. Thus the author focused in his present work to develop some possible color reactions to his chosen drugs to estimate them, by measuring their absorbance at various concentrations of drug samples at different wavelengths to establish linearity, precision, and accuracy by using Beers Lambert's law. The Chemical Structure of Bortezomib represents two secondary (**2**^{0}) & two tertiary (**3**^{0}) amines, two keto carbonyl groups and one borane gemdiols, So that the author expected oxidative coupling reactions due to the presence of **2**^{0} Amines, Ion exchange & ion association reactions due to presence of tertiary amines and also condensation reactions due to presence of carbonyl functional groups.

MATERIALS AND METHODS

Instruments

A **Shimadzu 2450** model, **0.1** nm resolution, double beam, **1cm** length quartz coated optics; Wavelength range**190-900nm**(performance guaranteed range) Extendable to **1100nm** through the use of photomultiplier. The UV Probe software gives you the power and versatility of an extremely sophisticated system in a package that's easy to learn and use. High stability, linearity, precision instrument is used for all the spectral measurements. All chemicals and reagents used in the analysis are of analytical grade and doubly distilled water is used for the preparation of all the solutions.

The chemicals and reagents used in the present study all are of analytical reagent grade. Bortezomib reference standard is kindly gifted by Chandra labs, Hyderabad. Pharmaceutical preparations containing Bortezomib capsules named as **VALCADE** contain 3.5 mg per 5 ml of injection formulation are purchased from Delhi.

Preparation of drug standard solution

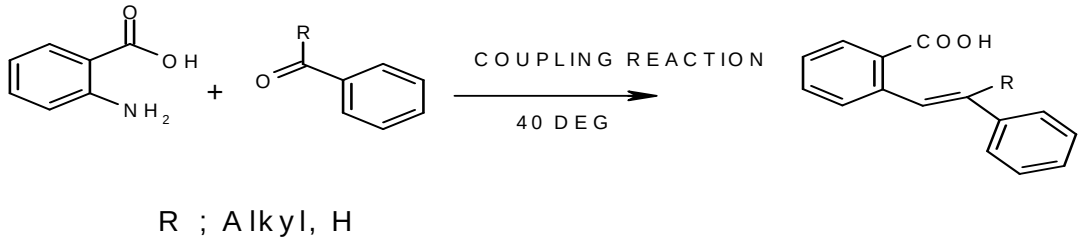
100 mg of the Bortezomib is accurately weighed and transferred into a 100 mL standard volumetric flask and dissolved in 10 ml 0.1 M Hydrochloric acid and this solution made up to the mark with approximately 90ml of distilled water with continuous shaking and working standards were prepared by diluting the standard drug stock solution in various levels like 20 µg/ml, 50 µg/mland100 µg/ml.

Chromo gens or Reagents

Author studied all the suitable reactions like DDQ, TQ, 4AP-NaIO4, Brucine and MBTH- Ce (IV) etc.., all responding to form a color complex, although 3 methods only forms a stable complexes .Hence those three color reagents used for estimation of bortezomib. They are **2-CPH** (Condensation reaction), **Anthranilic acid** (Condensation & Charge transfer reaction) and **MBTH-Iron III chloride** (Oxidative coupling reaction).

The amino group present in Anthranilic acid as shown as in figure -2 can condense with carbonyl groups (keto or aldehyde) thus this reaction is so called condensation reaction. Here in bortezomib we have two keto groups, hence it produced a color complex with Anthranilic acid. Here in estimation of bortezomib the author observed orange red colored stable complex with Anthranilic acid. For easy presentation purpose author represented this method as **Method-A**.

Reagent Mechanism:



Preparation of reagent-I:

Anthranilic acid (Aldrich; 0.25%) was prepared by dissolving 0.25 gm of Anthranilic acid in 100 ml of freshly distilled methanol.

Reagent-II: MBTH /Fe (III) Chloride

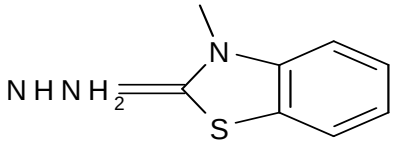
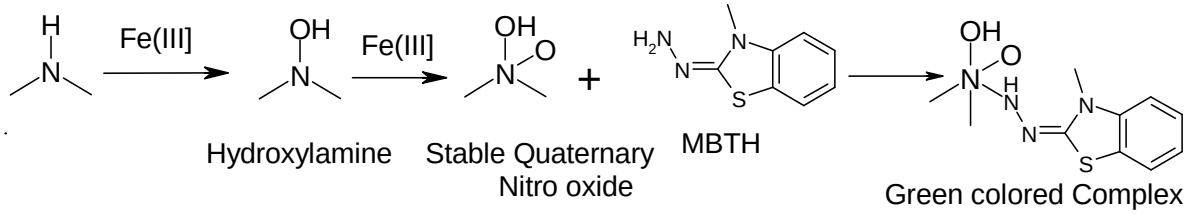


Figure-3: Structure of 3-Methyl-2-BenzoThaizolinone Hydrazone

Reagent Mechanism:



The secondary amine present in Bortezomib first Interact with 1 mole of Iron (III) chloride to form an unstable hydroxylamine derivative; which is further react with another 1mole of ferric chloride to form a stable Quaternary

Nitro-oxide (purple in color) and was finally subjected to oxidative coupling with MBTH then a stable **greenish brown colored complex** was observed. In the present thesis work this reagent was taken as **Method-B**

Preparation of Reagent-II
MBTH (Alfa Acer; 0.2%, 8.56x 10^{-3}M): It was prepared by dissolving 200mg of 3-methyl-2-Benzo Thaizolone hydrazone in 100 ml of doubly distilled water.

Iron (III) Chloride (Aldrich; 2%, 3.32x10^{-3}): Fe (III) solution is prepared by dissolving 2 gm of anhydrous ferric chloride in 100 mL of double distilled water, 2% (w/v)

Reagent-III: 2-Chloro Phenyl Hydrazine

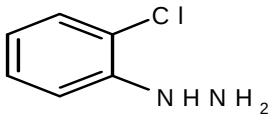
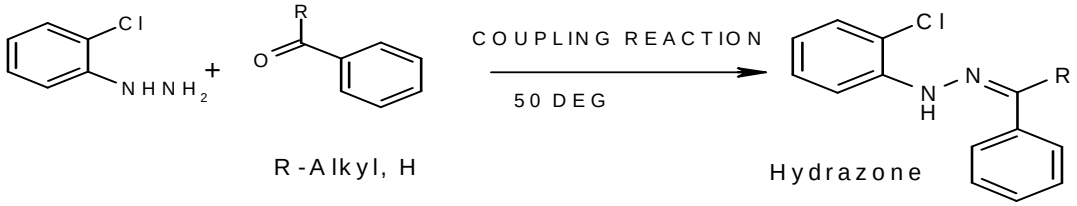


Figure-4: Structure of 2-Chloro Phenyl Hydrazine

Reaction Mechanism



The keto groups present in Bortezomib are directly interact with 2-Chlorophenylhydrazine in presence of an alcohol to form a **purpled colored complex** by the elimination simple water molecule, hence this reaction known as Condensation reaction and this method is represented as

Method-C.
Reagent preparation:
2-Chlorophnylhydrazine (Loba; 0.25%): This solution was prepared by dissolving 0.25 gm of 2-chlorophenylhydrazine in 100 ml methanol.

Experimental Procedure:
Method-A
Aliquots of (0.5 to 2.5ml, 50µg/ml) working standard solution of Bortezomib were transferred into a series of 10ml calibrated conical flasks. To each of above mentioned aliquots, 2.0 ml (0.25%) of anthranilic acid was added followed by one drop of concentrated hydrochloric acid and heat it to 40-45^0 C for half an hour. The solutions were made up to the mark with double distilled water. The absorbance is measured from 450 to 520 nm against a reagent blank prepared similarly and maximum absorbance is found at 485 nm (Fig.1). The amount of the BMB is calculated from its graph (Fig.3)

Method-B
Transferred (0.5 to 2.5 ml, 50 µg /ml) the working standard solution of BMB into a series of 10 ml calibrated conical flasks. To each of above aliquots, 2 ml of (0.25%) 2-chlorophenylhydrazine solution was added followed by 4 ml of 2% ethanolic potassium hydroxide and Heat it to 50^{0}C for 15 minutes. The solutions were made up to the mark with double distilled water. The absorbance is measured from 520-600 nm against a reagent blank prepared in the same way and absorbance maxima is found at 560 nm (fig-2). The amount of BMB is calculated from its graph (fig-4).

Method-C
Different portions (0.5-2.5 ml, 100 µg=1ml) of standard BMB solution is delivered into a series of 10 ml calibrated volumetric flasks. Added 1ml (2%) Iron (III) chloride solution followed by 2 ml of 0.2% MBTH reagent, and kept aside 5 minutes for the completion of reaction at the room temperature. The volumes were made up to the mark with double distilled water and the absorbance of each solution was measured from 595-665 nm against reagent blank.

The absorbance maxima is found at 630 nm (figure-333) and the calibration curve was prepared by plotting concentration versus absorbance and amount of drug was determined from its standard calibration curve.

RESULTS AND DISCUSSION

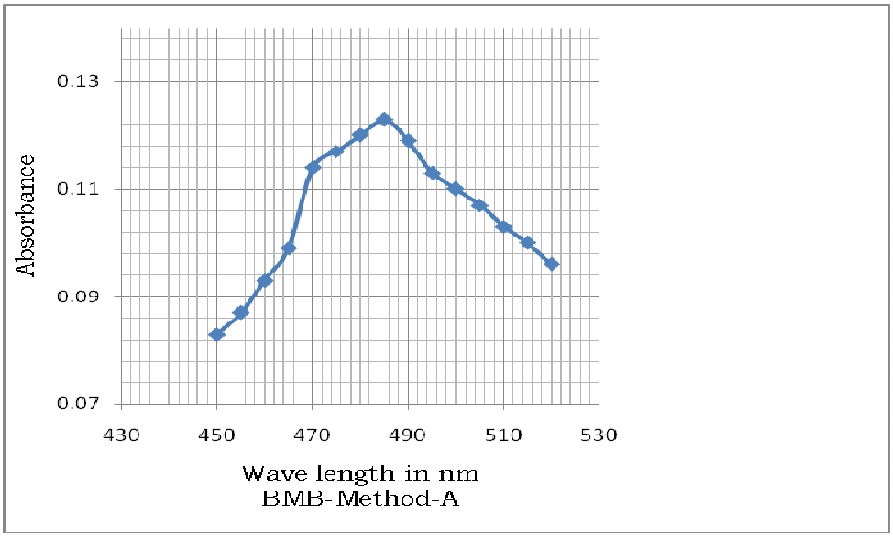


Figure-3: Beer's Law graph for BMB, (ATA, Method-A)

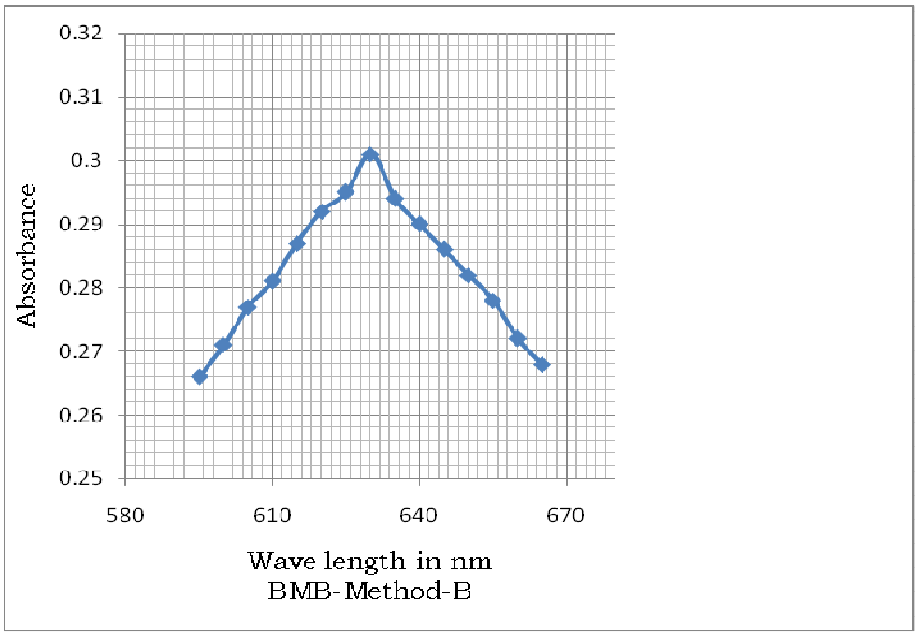


Figure-4: Beer's Law graph for BMB, (MBTH-Fe (III), Method-B)

In order to test whether the colored products formed in these methods adhere to Beer's law, the absorbencies at maximum wavelength of a series of six concentrations are plotted against concentration of the drug in µg/mL (Fig.6, Fig. 7, and Fig.8). Beer's law is obeyed within the limits 2.5-12.5 µg /mL 5-25 µg /mL and 2.5-12.5 µg /mL , molar absorptivity is found to be **4.630×10^4** , **1.544×10^5** and **7.76×10^4** for method-A, Method-B and Method-C respectively. Regression analysis of the Beer's law plots at Lambda max reveals a good correlation. The graphs show negligible intercept and are described by the regression equation, **$Y = b\,C + a$** (where ***Y*** is the absorbance of 1 cm layer, ***b*** is the slope, ***a*** is the intercept and ***C*** is the concentration of the measured solution in µg /mL). The high

molar absorptivites of the resulting colored complexes indicate the high sensitivity of the methods. Precision of the developed methods is ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of the test in total solution. The percent of relative standard deviation and percent range of error are calculated for the developed methods. To determine the accuracy of these methods, three different amounts of bulk samples within the linearity limits are prepared and analyzed by the developed methods. The percent recoveries of the drug by these methods are found to be within the range which indicates that the developed methods are accurate

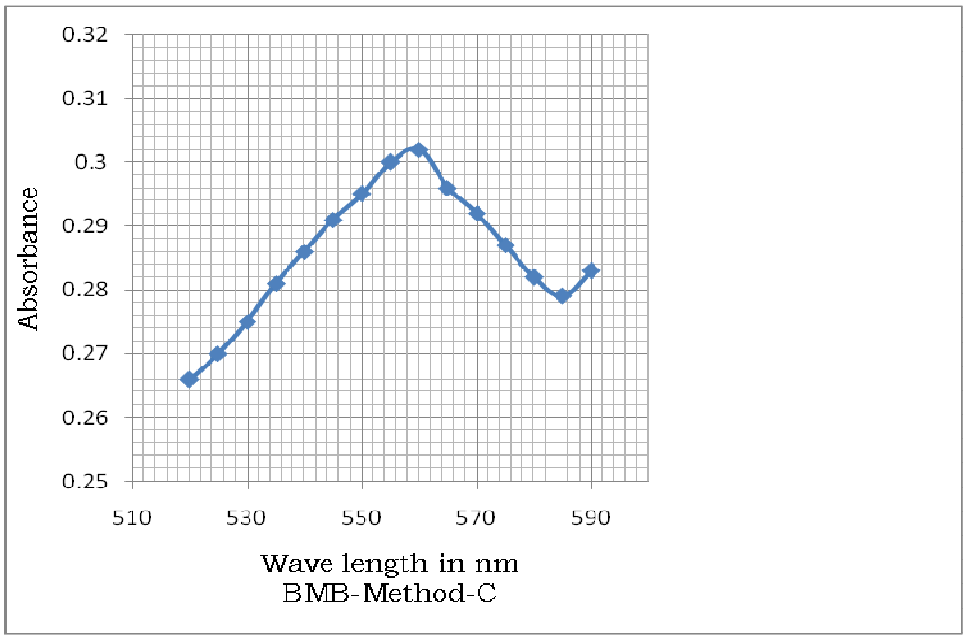


Figure-5: Beer's Law graph for BMB, (2-CPH, Method-C)

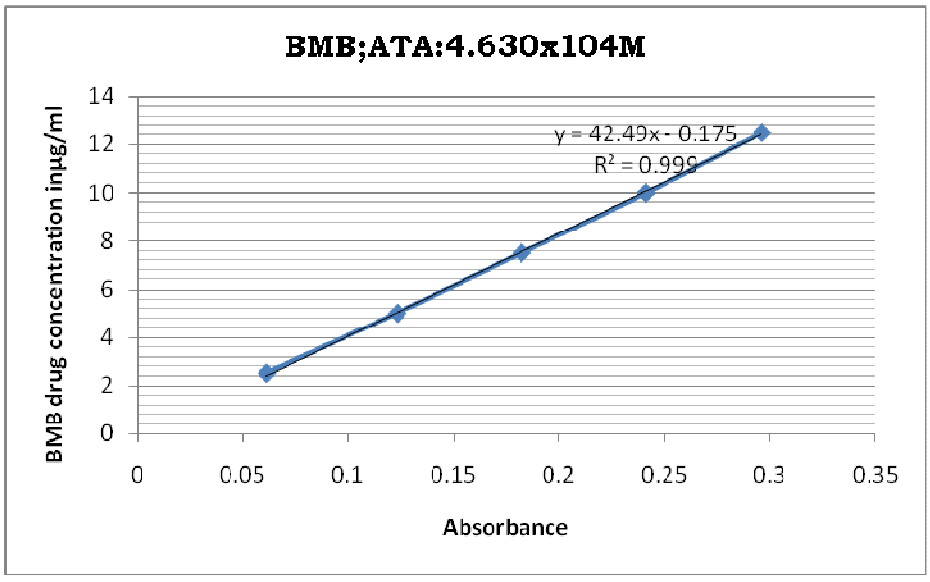


Figure-6: Linearity graph of Bortezomib with Anthranilic acid

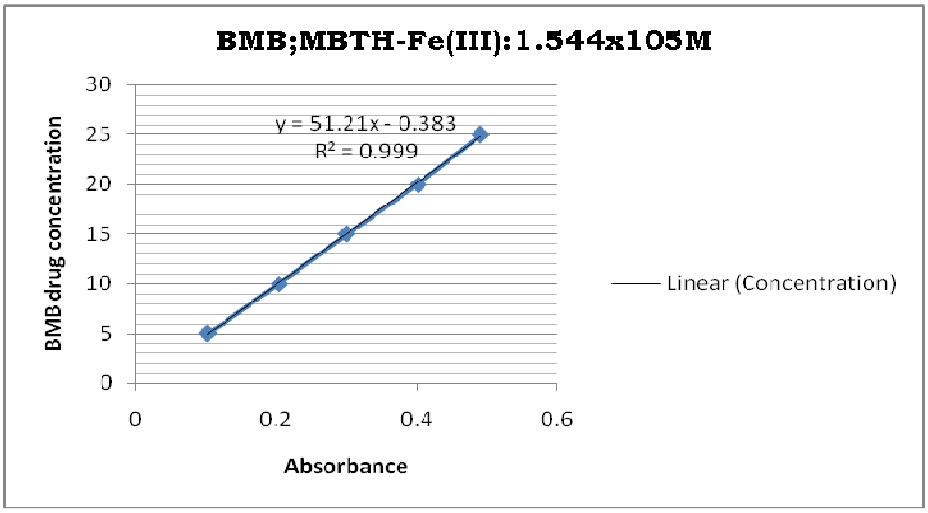


Figure-7: Linearity plot of Bortezomib with MBTH-Fe (III)

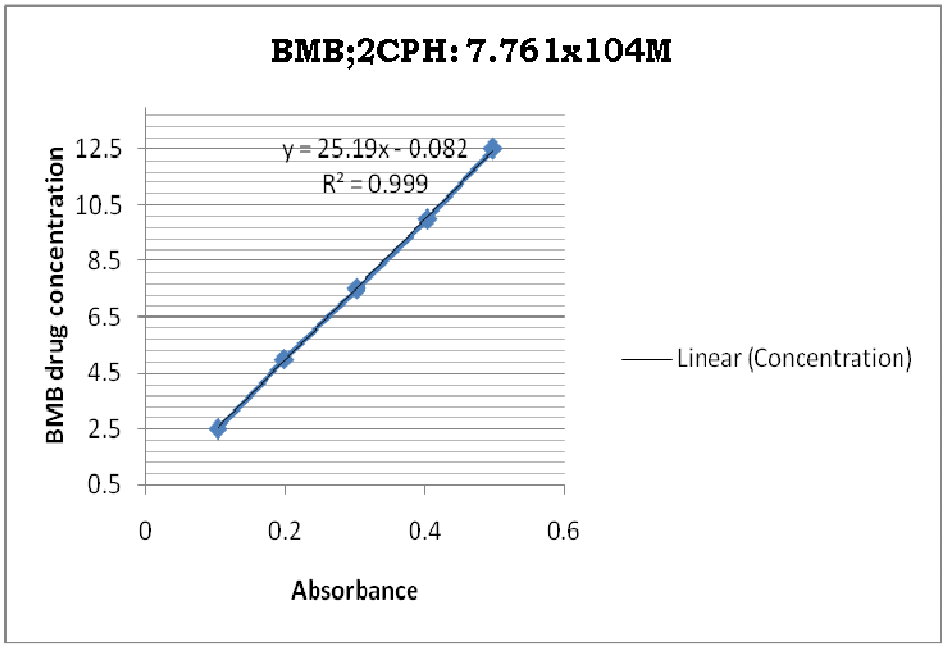


Figure-8: Linearity graph of Bortezomib with 2-CP

Table-1: Recovery of bortezomib in tablet by the proposed methods

Comparative Test results	Formulation Brand name	Labeled Amount	% Recovery Method-A	% Recovery Method-B	% Recovery Method-C
*	VALCADE	3.5 mg	99.1%	99.33%	99.69%
F-test	*	*	1.0678	1.2456	1.6231
t-test	*	*	0.9462	0.8924	0.5629

Each value is five determinations

Optical characteristics, linear regression parameters, precision, and accuracy of the proposed methods are presented in Table-1. These methods have been successfully applied for the determination of Bortezomib in pharmaceutical preparations. Bortezomib 3.5 mg was taken for the analysis. The percent of recovery of the drug is calculated and is compared with a reference method statistically by means of t-test and F-test at 95% confidence level and found the

developed methods are not significantly different. The results obtained by the developed methods are shown in Table-2.

Table-2; **Optical characteristics, Regression parameters, Precision and Accuracy of the proposed methods**

Parameter	Method-A	Method-B	Method-C
Maximum wave length (Lambda max)	485 nm	630 nm	560 nm
Beers Law limits (µg/ml)	2.5-12.5	5-25	2.5-12.5
Optimum Photometric Range(µg/ml)	0.2010	0.4425	0.2807
Sandell's Sensitivity (µg /cm2)	0.0207	0.02487	0.0123
Molar Absorptivity Lt/mole/cm	$4.630x10^4$	$1.544x10^5$	$7.761x10^4$
Slope (b)[a]	0.02352	0.0196	0.03968
Intercept(a)[a]	0.00420	0.0068	0.00340
Standard Deviation on slope(S_b)	0.000280	0.000174	0.000447
Standard Deviation on slope(Sa)	0.002329	0.002891	0.003713
Standard Error on Estimation(Se)	0.002221	0.002756	0.003540
Correlation Coefficient (r)	0.9997	0.9998	0.9998
Relative Standard Deviation	0.7688	0.5355	0.4636
Confidence limit @ 0.05	0.6425	0.4479	0.3877
Confidence limit @ 0.01	0.9503	0.6625	0.5735
Limit of Detection (LOD)µg/ml	0.3267	0.4867	0.3087
Limit of Quantification (LOQ)µg/ml	0.9902	1.4750	0.9359

[a] *Regression equation Y= a+bC ,Where Y stands for absorbance and C is concentration in µg/mL*
[b] *% Relative standard deviation is calculated for six determinations*

CONCLUSION

To summarize, our studies showed a possibility to use Anthranilic acid, MBTH-Fe (III) and 2-Chlorophenylhydrazine reagents for the spectrophotometric determination of Bortezomib. The determination procedures are characterized by Low detetection limit, Simple, reproducible and economic. The statical data the proposed methods are in good agreement with those of the known methods The colored species was stable for more than 5-6 hrs which is more sufficient for an analyst to perform the analysis. More over they do not required any pretreatment of the drug and tedious extraction procedures. Hence the data presented in the research paper by spectrophotometric methods is accurate and precise.

REFERENCES

[1] Vincent Chung,; Bingsen Zhou,; Xiyong Liu,; Lijun Zhu,; Lee M. Boo,; Ha-Van Nguyen,; David K. Ann4,; Jing Song, Yuan Chen and Yun Yen; *Mol Cancer Ther. March* **2006** *5;* 533,
[2] Zhen-Xian Du,; Hai-Yan Zhang,; Xin Meng,; Yifu Guan, and Hua-Qin Wang,; *BMC Cancer***. 2009**; 9: 56.
[3] Fahy BN,; Schliemann MG,; Virudachalam S et al.; *J Surg Res* **2003**; 113: 88–95.
[4] Gerald H. Manorek,; Alison K. Holzer; and Dr. Stephen B Howell; *Clin. Cancer Res*. January 15, **2009** 15: 553-560
[5] Cecilia Ceresa Elisa Giovannetti,; Jens voortman,; Adrie C Lana,; Richard Honeywell,; Giuseppe Giaccone and Godefridus J. Peters. *Mol Cancer* there may **2009**, 8, 1026
[6] J Scott Daniels, Teresa Pekol, Jasson Labutti, Lan Parsons, Darreil Nix, Elizibeth Baronas, Liang- Shnag Gan and Gerald Miwa *J. of drugmetobolism and Disproposition*, June **2005**, vol: 33, No: 6, 771-777.
[7] Jeffrey S. Johnston, Mitch A. Phelps, Kristine A. Blum, William Blum, Michael R. Graver, Katherine L. Farley and James T. Dalton, *Journal of chromatography*, vol: 871, August **2008,** issues 1, 15-21.