INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

A RAPID REVERSE PHASE HPLC DISSOLUTION METHOD DEVELOPMENT AND VALIDATION OF IMATINIB IN IMATINIB MESYLATE TABLETS DOSAGE FORM

SHAH P[1,3], SHAH N[2], BRAHMBHATT R[1], SAPTARSHI D[1], SHAH R[3]

1. Intas Pharmaceutical Limited-Astron Division, S. G. Highway, Bodakdev, Ahmedabad, Gujarat, India.
2. Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.
3. K K Shah Jarodwala Maninagar Science College, Gujarat University, Ahmedabad, Gujarat, India.

Accepted Date: 23/08/2014; Published Date: 27/08/2014

Abstract: A simple, rapid, accurate and isocratic reversed phase high performance liquid chromatographic dissolution method was developed and validate for the determination of Imatinib in tablet dosage form. The chromatographic separation was performed on ACE Phenyl (150mm x 4.6mm) 5µ column as stationary phase, using isocratic elution of buffer preparation (2.88 g of disodium hydrogen phosphate dissolve in 1000 ml of water and adjust pH of the buffer solution 2.70 pH with Ortho-phosphoric acid) and methanol mixed in a proportion of 400:600v/v as mobile phase. The flow rate was set at 1.0 ml/minute and UV detection was performed at 265 nm. The injection volume was 5 µl. The column oven temperature was maintained at 50°C. The Sample temperature was maintained at 25°C. The retention time of the Imatinib was found to be about 3.4 minute. The linearity of the drug was obtained in the concentration range of 20.000-120.000 µg/ml. All acceptance criteria of International Conference on Harmonization guideline for validation were covered in method validation. This method can be used for during manufacture in routine quality control sample and during real time stability sample analysis.

Keywords: Imatinib, HPLC, Method development, Validation, Dissolution.

Corresponding Author: MR. PRATIK SHAH

Access Online On:

www.ijprbs.com

How to Cite This Article:

Shah P, Shah N, Brahmbhatt R, Saptarshi D, Shah R; IJPRBS, 2014; Volume 3(4): 64-82

PAPER-QR CODE

INTRODUCTION

Imatinib mesylate is a tyrosine-kinase inhibitor used in the treatment of most particularly Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML), multiple cancers. The BCR-Abl tyrosine kinase enzyme exists only in cancer cells and not in healthy cells. Imatinib is a chemotherapy drug. It targets a specific protein within cancer cells and stops the cancer cells from growing and only cancer cells are killed through the drug's action. Imatinib mesylate was approved by the US food and drug administration to treat a rare cancer called chronic myeloid leukemia (CML)[1-3].

The chemical structure of Imatinib mesylate is given in figure 1. Imatinib mesylate is chemically known as 4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamid methane sulfonate with experiential formula $C_{29}H_{31}N_{7O}•CH_3SO_3H$. Imatinib mesylate molecular weight is 589.7 g/mol. Imatinib brand name is Gleevec or Glivec. The usual Imatinib mesylate tablet dosage is 100mg and 400mg. Imatinib is official in Indian Pharmacopoeia (IP) but Imatinib is not official in British Pharmacopoeia (BP) and United State Pharmacopoeia (USP). Literature survey reveals that several analytical methods like UV-spectrophotometric[4,5,6], HPTLC[7], RP-HPLC[8,9,10,11] methods are reported for estimation of Imatinib mesylate in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage form. The method was validated following the analytical performance parameters proposed by International Conference on Harmonization (ICH)[12].

Experimental

MATERIALS AND METHODS

Chemicals and Reagents

GR grade hydrochloric acid (Merck, India), AR grade disodium hydrogen phosphate (SDFCL, India), HPLC grade ortho-phosphoric acid (Spectrochem, India), HPLC grade methanol (Merck, India) were used. Working standard of Imatinib mesylate was with purity of 98.9%. Tablet formulations containing 100 mg and 400 mg of Imatinib mesylate tablets were prepared in laboratory as a process of developing finished product. Standard and samples were provided by Intas Pharmaceutical Limited-Astron Division, Gujarat, India. The nylon filters with pore size of 0.45µ were used to filter solutions.

Instrumentations

Analysis was performed on Agilent HPLC 1100/1200 series system, consisting of quaternary pump, auto sampler, UV detector and DAD detector. The output signal was monitored and processed by Chromeleon software. Calibrated dissolution test apparatus (TDL-08L) of electro

lab, analytical balance Mettler Toledo (Model: AG204, AG245), analytical balance Precisa (Model: XR205SM-DR, BJ 610C), media preparator (Model: EMP-21) of Electro lab, pH meter of Lab india (Model: Pico+) and ultra sonic cleaner of equitron were also used during the analysis.

Chromatographic parameters

The analytical column was ACE Phenyl (150 mm x 4.6 mm) 5µ Make: ACE as stationary phase. The flow rate was maintained at 1.0 mL/minute. The detection was performed at a wavelength of 265 nm. The column oven temperature was maintained at 50°C and sample temperature was maintained at 25°C. The injection volume was 5 µl. The run time of standard and sample was 5 minutes. The retention time of Imatinib peak was about 3.4 minute. Dissolution medium as diluent use.

Dissolution parameters

Dissolution medium volume was 1000 ml with 0.01N hydrochloric acid in water. Dissolution apparatus was used USP Type II (Paddle). Dissolution speed was 50 RPM. Dissolution time was 45 minutes. Dissolution temperature was 37 ± 0.5°C.

Preparation of dissolution medium (0.01N hydrochloric acid)

Dilute 8.5 mL of hydrochloric acid (35%) to 10000 mL with water.

Buffer preparation

Weigh accurately about 2.88 g of disodium hydrogen phosphate and dissolve it in 1000 mL of water. Adjust the pH of the solution to 2.70 pH with ortho-phosphoric acid.

Mobile phase

Prepare a mixture of 400 volumes of buffer preparation and 600 volumes of methanol. Filter through 0.45 µ filter and degassed before use.

Standard preparation

Transfer an accurately weighed quantity equivalent to 10.0 mg of Imatinib (about 11.95 mg of Imatinib mesylate) working standard in to 100 mL volumetric flask. Dissolve and dilute to volume with diluent and mix well.

Sample preparation (for 100 mg)

Set dissolution parameters. Fill the dissolution bowls with the mention quantity of dissolution medium. Drop six tablets in to 6 separate dissolution bowls taking care to exclude air bubbles

from the surface of the tablet and immediately start the apparatus. After 45 minutes withdraw the sample of medium and filter through 0.45µ nylon filter. Discard first few mL of the filtrate.

Sample preparation (for 400 mg)

Sample preparation for 400 mg refer same as sample preparation for 100 mg and further dilute 5.0 ml of this solution to 20 ml with diluent and mix well.

Method validation

The proposed HPLC method for determination of dissolution of Imatinib in Imatinib mesylate tablet is in-house developed and is to be validate as per current ICH guideline. Formulation of Imatinib mesylate tablets 100 mg and 400 mg are step up and step down formulation. Hence, the entire method validation study has been planned on lower strength i.e.100 mg and it is applicable for all other strengths also. The various performance of method validation parameters like specificity, stability of analyte in solution, system precision, method precision, intermediate precision, linearity, accuracy and robustness to be evaluated to be prove the performance of any newly developed method before adopting it for routine use. Method validation covers all parameters and acceptance criteria defined in international conference harmonization guideline for analytical method validation.

Specificity

Prepare single set of placebo preparation (mentioned as below) containing all inactive ingredients in the same proportion as in the formulation, standard preparation and sample preparation as per method. Inject single injection of diluent, placebo preparation, standard preparation and sample preparation into the liquid chromatograph and record the chromatograms. Identify the peaks in the HPLC chromatogram coming from diluent and placebo preparation. Check for interference of the diluent and placebo peaks at the retention time of Imatinib peak. Identify the peak due to Imatinib in the sample preparation based on retention time obtained in standard preparation.

Placebo Preparation: Set dissolution parameters. Fill the dissolution bowl with the mention quantity of dissolution medium. Place about 75.5 mg of placebo powder in to vessel and lower down the hood and immediately start the apparatus. After 45 minutes, withdraw a sample from glass vessel and filter the solution immediately through 0.45µ nylon filter. Discard first few ml of the filtrate.

Stability of analyte in solution

Perform stability studies of standard preparation and sample preparation at 23°C to 27°C and not protected from light. Prepare standard and sample preparation as per method. Store them on bench top 23°C to 27°C and not protected from light. Measure the respective peak area of the stored solution at different time intervals at about initial, 6 hours, 12 hours, 18 hours and 24 hours. Calculate the percentage difference in peak area of standard preparation and sample preparation at different time intervals in comparison to the initial respective peak area. Perform solution stability at 23°C-27°C and protected from light, 2°C-8°C protected from light in sub sequentially if it is failed at 23°C-27°C and not protected from light.

Linearity

Prepare different linearity level solutions of Imatinib working standard to achieve concentrations ranging from 20% to 120% of test concentration for Imatinib and 100% linearity level considering standard preparation concentration 100.000 µg/ml. Prepared different linearity level solutions in diluent with final concentrations from 20.000 µg/ml, 50.000 µg/ml, 70.000 µg/ml, 100.00 µg/ml, 110.000 µg/ml, 120.000 µg/mL of working concentration. Inject single injection of all linearity solutions in to liquid chromatographic system and record the chromatograms. Plot a linearity curve of concentration in µg/mL verses peak area. Calculate the response factor for each concentration level. Calculate correlation coefficient, slope of regression line, Y-intercept, R^2 value and %Y-intercept bias at 100% level. Also calculate the relative standard deviation of response factor for each concentration level.

Precision

System precision

Prepare a standard preparation as per method. Inject six replicate injections of the standard preparation into the liquid chromatographic system and record the chromatograms. Check the system suitability as per method.

Method precision

Carry out dissolution for six individual tablets as per method. Calculate the dissolved quantity of Imatinib in terms of percentage of label claim against standard preparation. Determine the mean and relative standard deviation of test results.

Intermediate precision

Perform the method precision study using different HPLC, different dissolution apparatus, different column (same make but different lot no.) and different analyst on a different day.

Calculate the mean and percentage relative standard deviation of six results. Calculate the absolute difference and similarity factor f_2 value between the six results obtained between method precision study and intermediate precision study.

Accuracy

Prepare accuracy samples in triplicate set in the range of 20% to 120% of test concentration and perform the dissolution as per method.

Preparation of accuracy stock solution: Transfer an accurately weighed quantity equivalent to 1000.0 mg of Imatinib (about 1210.0mg of Imatinib mesylate) working standard in to a 250 ml volumetric flask. Dissolve and dilute to volume with diluent and mix well. (Concentration: 4.0mg/ml of Imatinib)

Set dissolution parameters. Fill the dissolution bowls with the above mentioned quantity of dissolution medium, add placebo and accuracy stock solution for each level as stated in the above Table-1 in three separate dissolution bowls for each level and immediately start the apparatus. After 45 minutes withdraw the sample of medium and filter through 0.45µ nylon filter. Discard first few ml of the filtrate.

Inject single injection of all triplicate preparations in to the liquid chromatographic system and record the chromatograms. Calculate the mean percentage accuracy, relative standard deviation for each level. Also calculate the overall percentage accuracy and relative standard deviation for all the results.

Robustness

To determine the robustness of the analytical method, experimental conditions were deliberately altered. Influence of small changes in chromatographic conditions were studied by testing effect of minor variation in column oven temperature (±2°C) (i.e. 48°C and 52°C), variation in the flow rate (±0.2 ml/minute) (i.e. 0.8 ml/minute and 1.2 ml/minute), variations in mobile phase buffer pH (±0.2 pH) (i.e. 2.5 pH and 2.9 pH) and variation in mobile phase composition (± 5%) (i.e. ±5% of methanol). The change in method parameters were studies by testing effect of minor variation in temperature of dissolution medium (± 2°C) (i.e. 35°C and 39°C), variation in speed of dissolution apparatus (± 2 RPM) (i.e. 48 RPM and 52 RPM), variation in media volume (± 5%) (i.e. 950 mL and 1050 mL), variation in mode (auto sampler) and without dissolution media degassing. Calculate the percentage dissolved in each case. Calculate the absolute difference between the results obtained by the varied method and the method precision results.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic parameters

Development of dissolution method of Imatinib tablets in which Kromasil 100-5 C18 (250mm X 4.6mm), 5µ Make: Akzonobel column was used at flow rate 1.0 ml/minute. The column oven temperature was 30°C and injection volume 20 µl was injected with mobile phase ratio of buffer preparation (dissolved 2.88 gm of disodium hydrogen phosphate in 1000 ml of water and adjust pH 2.70 with diluted ortho-phosphoric acid. Filter through 0.45µ filter) : methanol mixed in a proportion of 400:600v/v. Maxima of imatinib observed at 265 nm in which retention time of imatinib peak observed at 3.5 minutes. Hence, run time was decrease because Imatinib peak eluted at 3.5 minutes. In which peak shape was not good in acetate and phosphate buffer. Hence, decrease injection volume for decrease column load and increase column oven temperature 50°C is helpful for better peak shape also change buffer acidic side for better peak shape in acetate and phosphate buffer. Finally, Kromasil 100-5 C18 (250mm X 4.6mm), 5µ Make: Akzonobel column was replaced by ACE Phenyl (150mm X 4.6mm), 5µ due to good peak shape and increase sample concentration for increase peak area. In which retention time of Imatinib was found at 3.5 minutes. Also theoretical plate, tailing factor are well within limit. Hence, this method was recommended for validate and use for routine dissolution analysis for Imatinib tablets.

Results for method validation parameters

Specificity

No interference is observed at the retention time of Imatinib peak due to diluent and placebo preparation. Peak obtained due to Imatinib at the retention time of 3.257 minute in standard preparation (Figure 2). Peak obtained due to Imatinib at the retention time of 3.260 minute in sample preparation (Figure 2).

Stability of analyte in solution

Percentage difference of peak area of Imatinib peak is well within the limit for standard and sample preparation from initial respective area up to 24 hours at 23°C-27°C and not protected from light. Standard and sample preparations are stable up to 24 hours at 23°C-27°C and not protected from light (Table-2).

Linearity

The correlation coefficient is 0.99991. Percentage relative standard deviation of response factor is 0.98. Percentage Y-intercept bias at 100% level is -0.5 (Table-3 and Figure 3).

Precision

System Precision (System suitability)

System suitability passes as per acceptance criteria (Table-4).

Method precision

Percentage relative standard deviation of six results is well within the limit. Results show good degree of precision (Table-5).

Intermediate Precision

Percentage relative standard deviation of six results is well within the limit. Percentage difference in average results obtained between method precision and intermediate precision is well within the limit. Similarity factor f_2 value is well within the limit (Table-5).

Accuracy

Percentage accuracy of Imatinib is well within the limit. Percentage relative standard deviation for accuracy at each level is well within the limit. Over all percentage relative standard deviation for all the levels is well within the limit (Table-6).

Robustness

There was no significant impact on the retention time of Imatinib peak, theoretical plates and tailing factor. Comparison of system suitability between system precision and altered method parameters are given Table-7. Comparison of results of sample preparation for method precision and altered robustness parameters are given Table-8.

CONCLUSION

A rapid isocratic RP-HPLC dissolution method with UV detection was developed for the determination of Imatinib. The run time of this method is relatively shorter 5 minute with better solution stability for minimum 24 hours allows analysis of many samples per day in comparatively low cost. Method validation results has proven that the method is specific, rapid, precise, accurate, linear and robust.

ACKNOWLEDGEMENT

The authors are thankful to Dr Rutesh R Shah, Dr Nisha K Shah, Riddhesh H Brahmbhatt and Dipak Saptarshi for their encouragement and support to complete this work. The authors are

also thankful to Intas Pharmaceutical Limited-Astron Division for providing all the essential facilities to carry out this work successfully.

AUTHOR'S STATEMENT

The author declare that we have no conflict of interest.

Table-1 Accuracy

Accuracy level	Volume of dissolution medium in mL	Weight of placebo to be taken in mg	Volume of Accuracy stock solution to be taken in mL	Theoretical amount of Imatinib in final sample preparation in µg/mL
20 %	995	75.5	5	20.0
100 %	975	75.5	25	100.0
120 %	970	75.5	30	120.0

Table-2 Stability of analyte in solution

Time interval	For standard preparation	For sample preparation
	%Difference	%Difference
Initial	NA	NA
6 hours	0.31	0.10
12 hours	0.43	0.57
18 hours	0.35	0.41
24 hours	0.20	0.49

Table-3 Linearity

Linearity levels	Concentration of Imatinib mcg/mL	Peak Area	Response Factor
20%	20.000	390.43854	19.522
50%	50.000	997.52525	19.951
70%	70.000	1388.66898	19.838
100%	100.000	1981.95194	19.820
110%	110.000	2212.05052	20.110
120%	120.000	2390.04256	19.917
%RSD of response factor:			0.98
Correlation Coefficient:			0.99991
R^2 value:			0.99982
Y-Intercept:			-9.67266
Slope:			20.03982
% Y-Intercept bias at 100% level:			-0.5

Table-4 System precision

System suitability parameters	Observation	Acceptance criteria
%RSD for peak area of Imatinib from replicate injection of standard preparation.	0.15	NMT 2.00
Mean theoretical plates for the Imatinib peak in standard preparation.	7512	NLT 1000
Mean tailing factor for the Imatinib peak in standard preparation.	1.10	NMT 2.00
Mean retention time for Imatinib peak in minutes	3.260	Not applicable
Difference in retention time for Imatinib in standard preparation.	0.0 minute	±0.2 minute from average retention time

Tablel-5 Comparison between method precision and intermediate precision

Tablet No.	Method Precision	Intermediate Precision
1	97.9	98.5
2	98.5	98.1
3	97.2	98.2
4	99.7	98.0
5	99.4	99.1
6	98.1	98.4
Mean	98	98

%RSD	0.96	0.40
Absolute difference		0.0
f_2 Value		94

Table-6 Accuracy

Set	Accuracy level	Practical amount in µg/mL	Theoretical amount in µg/mL	% Accuracy
Set-1	20%	19.805	19.999	99.0
Set-2		20.001	19.999	100.0
Set-3		19.921	19.999	99.6
Mean				99.5
%RSD				0.49
Set-1	100%	100.003	99.961	100.0
Set-2		99.983	99.961	100.0
Set-3		99.754	99.961	99.8
Mean				100.0
%RSD				0.14
Set-1	120%	119.775	119.953	99.9
Set-2		119.954	119.953	100.0
Set-3		120.011	119.953	100.0
Mean				100.0
%RSD				0.10
Overall Mean				99.8
Overall %RSD				0.33

Tablet-7 Comparison of system suitability between system precision and altered Method parameters

Robustness conditions	%RSD of peak area	Theoretical plates	Tailing factor	Mean retention time (minute)	Difference in retention time (minute)
System precision (Precision study)	0.15	7512	1.12	3.260	0.0 minute
Flow rate: 0.8 ml/minute	0.18	7311	1.11	4.157	0.0 minute
Flow rate: 1.2 ml/minute	0.24	7054	1.08	2.790	0.0 minute
Column oven temperature: 48°C	0.15	6798	1.06	3.392	0.0 minute
Column oven temperature: 52°C	0.19	6215	1.12	3.242	0.0 minute
pH of mobile phase buffer: pH 2.5	0.21	6454	1.14	3.244	0.0 minute
pH of mobile phase buffer: pH 2.9	0.08	6837	1.09	3.398	0.0 minute
Mobile phase composition: -5% Methanol	0.09	6201	1.07	3.655	0.0 minute
Mobile phase composition: +5% Methanol	0.17	6359	1.14	2.872	0.0 minute
Acceptance criteria	Not more than 2.00	Not less than 1000	Not more than	Not applicable	±0.2 minute

	2.00		from average retention time

Tablet- 8 Comparison of results of sample preparation for Method precision and altered Robustness parameters

Robustness parameters	Mean	% RSD	Absolute difference
Without Variation (Method precision)	98	0.96	NA
Variation in speed of dissolution apparatus: 48 RPM	99	1.36	1.0
Variation in speed of dissolution apparatus: 52 RPM	98	1.21	0.0
Variation in dissolution medium temperature: 35°C	98	1.56	0.0
Variation in dissolution medium temperature: 39°C	99	2.54	1.0
Variation in dissolution medium volume: 950 mL	99	2.22	1.0
Variation in dissolution medium volume: 1050 mL	98	2.51	0.0
Auto sampling in dissolution	99	1.75	1.0
With dissolution medium degassing	98	2.42	0.0
Variation in mobile phase buffer pH 2.5	99	1.75	1.0
Variation in mobile phase buffer pH 2.9	99	1.63	1.0
Variation in organic solvent concentration (-5% methanol) buffer: methanol (430:570)	100	1.55	2.0
Variation in Organic solvent concentration (+5% methanol) buffer: methanol (370:630)	99	1.31	1.0
Acceptance criteria	Not applicable	Not more than 6.00	Not more than 5.0

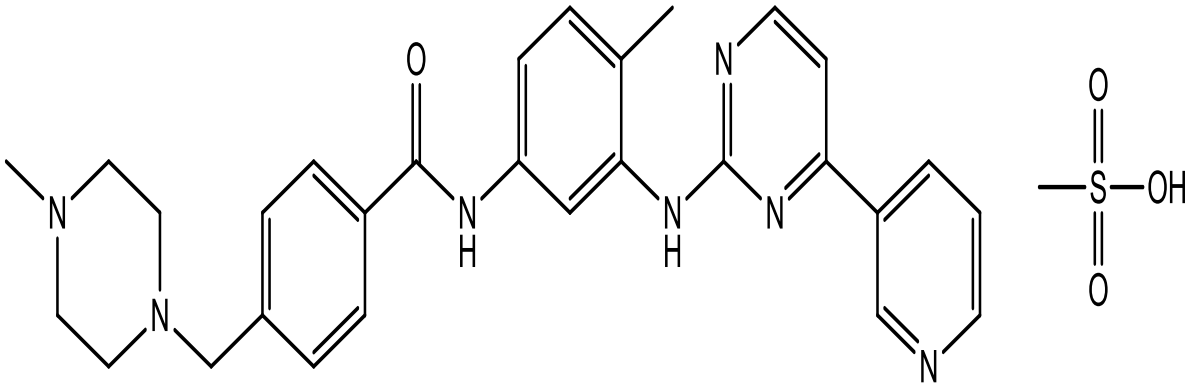
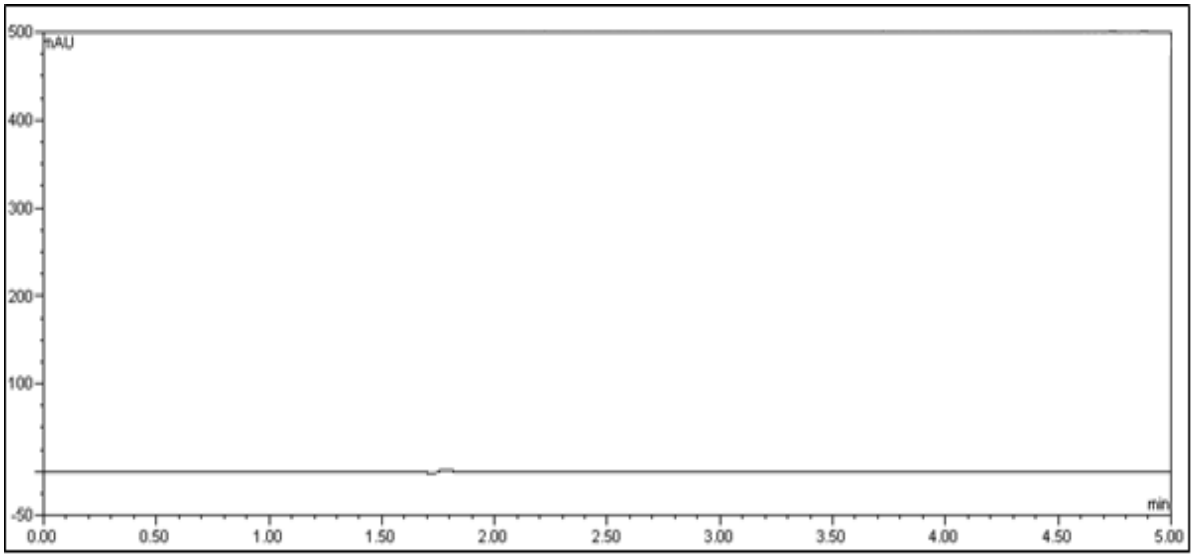
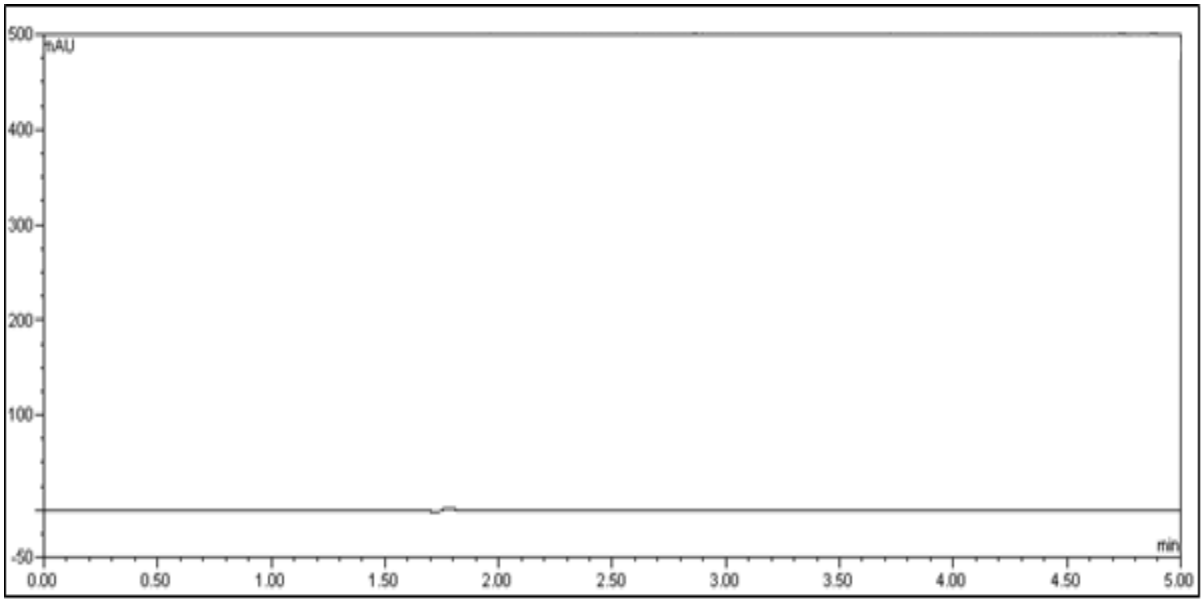


Figure 1: Imatinib mesylate

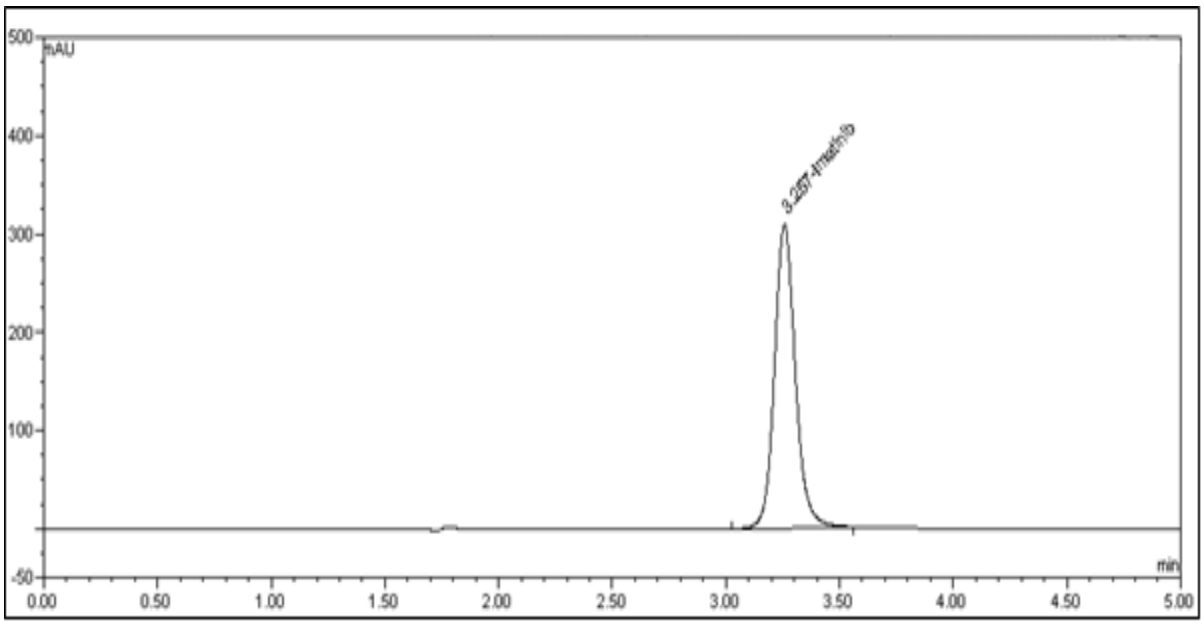
(A)


mAU
500
400
300
200
100
-50
min
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00

(B)


mAU
500
400
300
200
100
-50
min
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00

(C)



(D)

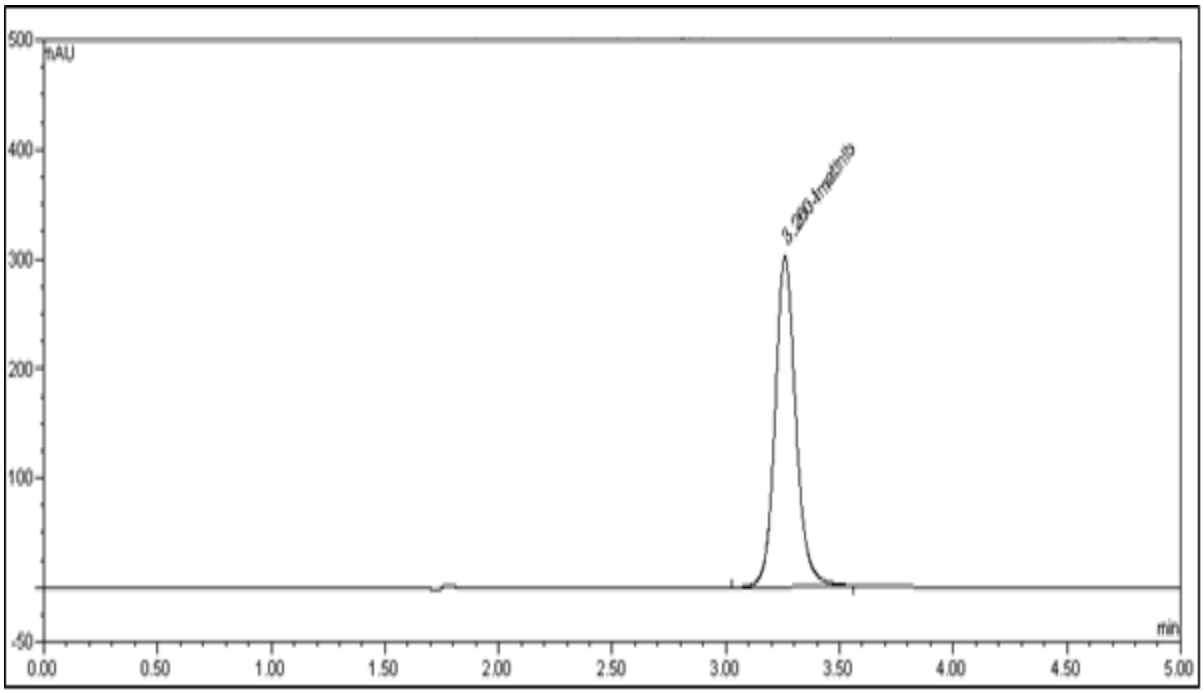


Figure 2: Chromatograms for specificity (A) Diluent (B) Placebo preparation (C) Standard preparation (D) Sample preparation.

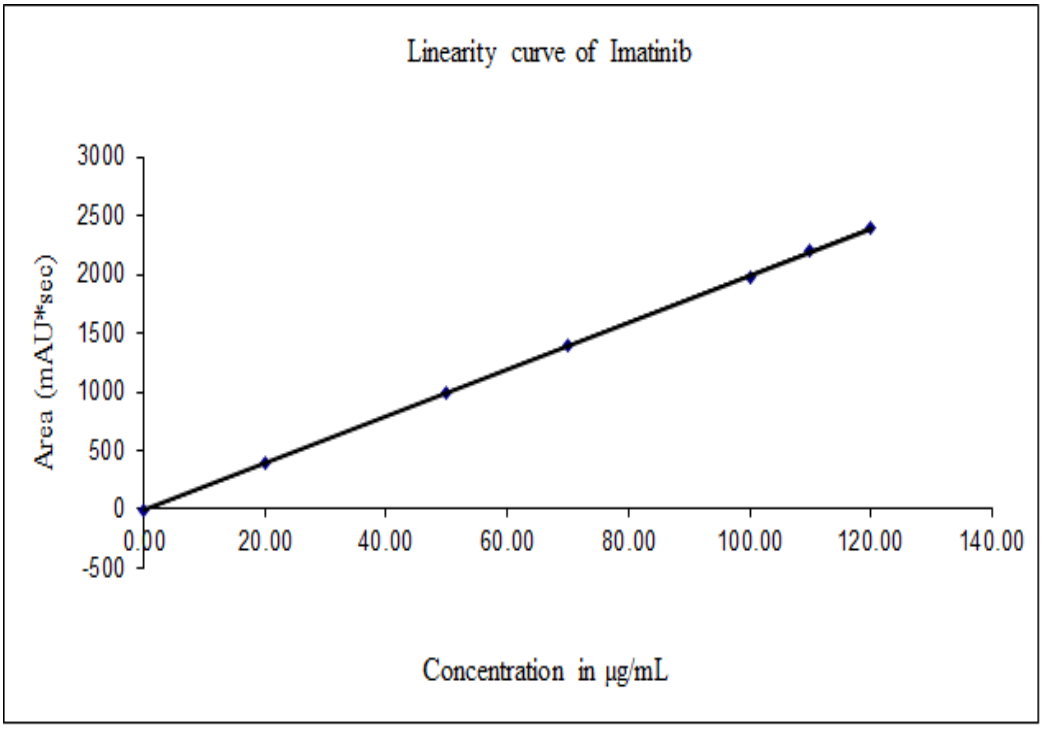


Figure 3: Linearity curve for Imatinib

REFERENCES

1. Imatinib-Wikipedia, (http://en.wikipedia.org/wiki/Imatinib). Accessed 03 February 2014

2. Fausel C: Targeted chronic myeloid leukemia therapy: Seeking a cure. American journal of health system pharmacy 2007; 64: S9-15.

3. Stegmeier F, Warmuth M, Sellers WR and Dorsch M: Targeted cancer therapies in the twenty-first century: lessons from Imatinib. Clinical pharmacology and therapeutics 2010; 87: 543–552.

4. Patil SJ, Doijad RC and Dhumal PP: Development of UV-Spectrophotometric Method for determination of Imatinib Mesylate in Bulk & Formulation. Asian Journal of Pharmaceutical and Clinical Research 2013; 6: 54-57.

5. Bende G, Kollipare S, Sekar V and Saha R: UV-Spectrophotometric determination of Imatinib mesylate and its application in solubility studies. Pharmazine 2008; 63: 641-645.

6. Kumar RJ and Kumar SN: Validated Spectrophotometric estimation of Imatinib Mesylate in pure & tablet dosage form. International Journal of Pharmacy & Technology 2010; 2: 490-495.

7. Vadera N, Subramanian G and Musmade P: Stability indicating HPTLC determination of Imatinib Mesylate in Bulk drug & Pharmaceutical dosage. Journal of pharmaceutical and biomedical analysis 2007; 43: 722-726.

8. Kuna A and Kuna J: RP-HPLC method Development & Validation of Imatinib Mesylate in tablet dosage form. International Journal of Pharmacy & Pharmaceutical Sciences 2011; 3: 162-165.

9. Aleti P, Venisetty RK and Kamarapu SK: Development of RP-HPLC method for analysis of Imatinib Mesylate using PDA Detector & its application in the evaluation of marketed preparation. International Journal of Pharmacy and Biological Sciences 2011; 1: 14-18.

10. Negi LM, Jaggi M and Talegaonkar S: Development of High Sensitivity RP-HPLC method & Stress Testing of Imatinib Mesylate. Journal of Advanced Pharmacy Education & Research 2013; 3: 238-243.

11. Maria AR, Maria AM, Maria TP and Adriana IS: Validation of an HPLC Method for the Determination of Imatinib Mesylate in Pharmaceutical dosage. Journal of Liquid Chromatography & Related Technologies 2013; 28: 3283-3292.

12. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005.