IJPSR (2015), Vol. 6, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 April, 2015; received in revised form, 09 June, 2015; accepted, 22 August, 2015; published 01 October, 2015

METHOD DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR ASSAY DETERMINATION OF IMATINIB IN IMATINIB MESYLATE TABLETS DOSAGE FORM

Pratik Shah[1, 3], Nisha Shah [2] and Rutesh Shah[*3]

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

Keywords:

Method development, Validation,Stability Indicating, RP-HPLC, Assay, Imatinib.

Correspondence to Author:
Rutesh Shah

Principal,
K. K. Shah Jarodwala Maninagar Science College, Gujarat University, Ahmedabad, Gujarat, India.

E-mail: dr.ruteshrshah2304@gmail.com

ABSTRACT: A simple, rapid and isocratic reversed phase high performance liquid chromatographic (RP-HPLC) stability indicating method was developed and validated for the determination of Imatinib in tablet dosage form. Imatinib drug was exposed to several stress conditions. The proposed method proved to be stability indicating by forced degradation experiments and mass balance study. The chromatographic separation was achieved using Eclipse XDB-C18 (150 mm X 4.6 mm) 5µ analytical column as the stationary phase with isocratic elution of the mobile phase composition of 1.5 g of Sodium dihydrogen phosphate in to 500 ml of water and adjust pH 8.00 with Triethylamine (buffer preparation) : pepared a mixture of 300 ml volumes of methanol and 200 ml volumes of acetonitrile (solvent mixture) mixed in a proportion of 450:550v/v at a flow rate of 1.0 ml/minute. The column oven temperature was maintained at 50°C. The Sample temperature was maintained at room temperature. The injection volume was set to 20 µl and the detector was performed at 265 nm. The retention time of the Imatinib was found to be about 5.5 minute. The linearity concentration range was 19.815-29.722 µg/ml with correlation coefficients 0.99990. The developed method was validated in accordance with ICH guidelines.

INTRODUCTION: Imatinibme sylate is a tyrosine-kinase inhibitor used in the treatment of most particularly "Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia" (CML) and multiple cancers. Imatinibacts by inhibiting BCR-Abl, a type of tyrosine-kinase, from phosphorylating subsequent proteins and starting the signalling cascade necessary for preventing the growth of cancer cells and leading to their death by apoptosis.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(10).4453-68

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(10).4453-68

The BCR-Abl tyrosine kinase enzyme exists only in cancer cells and not in healthy cells. Imatinib is a chemotherapy drug. It is mainly used to treat certain leukemias, myelodysplastic syndromes and other cancers. It is also used in the treatment of specific digestive tract tumors called gastrointestinal stromal tumors (GISTs). Imatinib is one of the first anticancer drug to show the potential for such a targeted action and is often cited as a paradigm for research in cancer therapeutics. [1-3]

The chemical structure of Imatinib mesylate is shown in **Fig. 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 and

has a chemical formula $C_{29}H_{31}N_7O{\bullet}CH_3SO_3H$. A molecular weight of Imatinib mesylateis 589.7 g/mol. Imatinib mesylate is a white to creamish yellow crystalline powder. It is freely soluble in distilled water, 0.1N HCl and methanol. Imatinibmesylate was approved by the US food and drug administration to treat a rare cancer called chronic myeloid leukemia (CML). Imatinib is being sold under the brand name "Gleevec" or "Glivec". Imatinibmesylate tablets are usually given in 100mg and 400mg dosage forms. Imatinib is officially registered in Indian Pharmacopoeia (IP). Several analytical methods like UV-spectrophotometric [4-6], HPTLC [7]and HPLC [8-13] have been reported in the literature for estimation of Imatinib mesylate in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage form.

There are three main impurities, Impurity A, B and C, known for Imatinib are shown in **Fig. 2, 3** and **4** respectively. Impurity A is chemically known as 4-(4-Methyl-1-piperazinomethyl) benzoic acid dihydrochloride; Impurity B as N-(5-Amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine amine and Impurity C as 4-[(Piperizinyl)methyl]-N-[4-Methyl-3-[(4-pyridinyl)-2-pyrimidinyl amino] phenyl] benzamide.

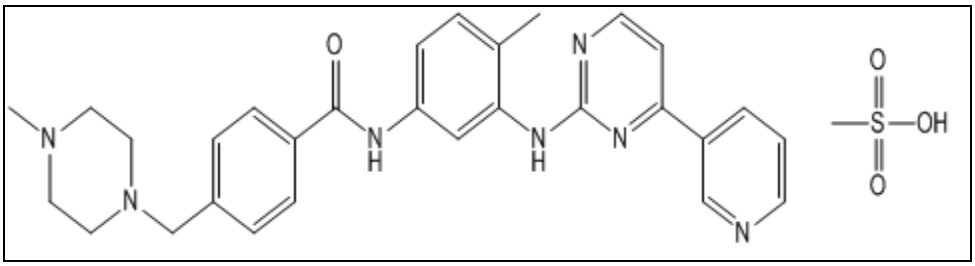


FIG. 1: IMATINIB MESYLATE

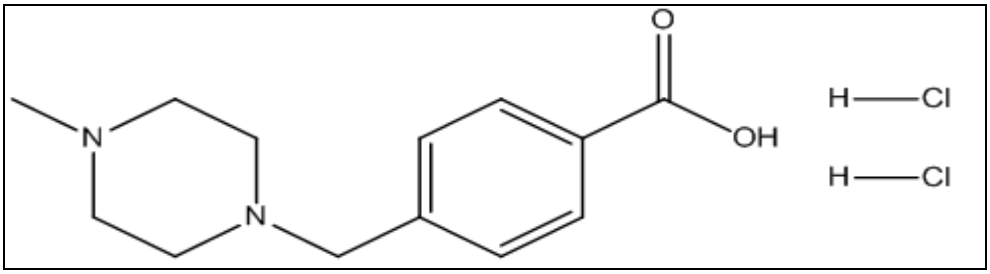


FIG.2: IMPURITY A

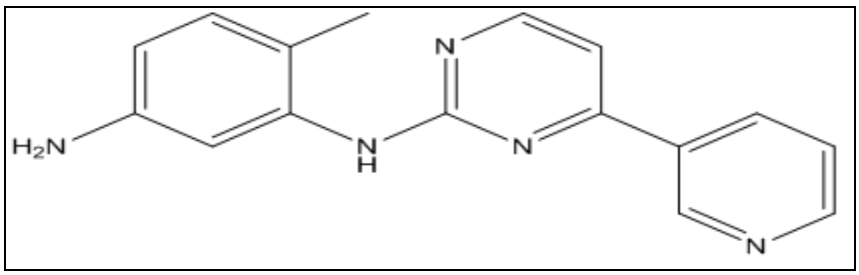


FIG.3: IMPURITY B

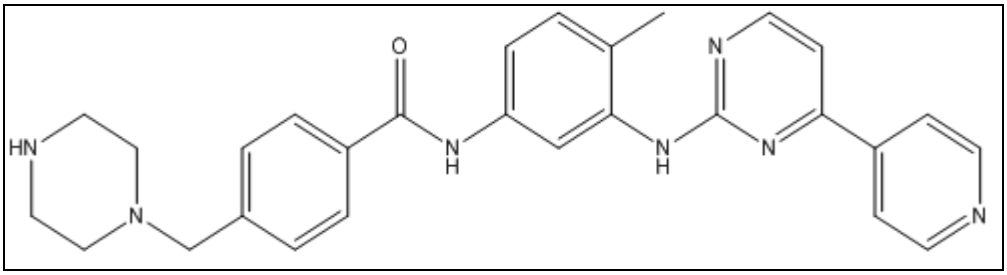


FIG.4: IMPURITY C

Validation of a method is the process by which a method is tested by the developer for reliability, accuracy and preciseness of its intended purpose. Validation of analytical method is the process of establishing a method which can meet its intended analytical requirement. Various validation parameters like specificity, stability of analyte in solution, linearity, system precision, method precision, intermediate precision, accuracy and robustness need to be evaluated to prove the performance of any newly developed method before adopting it for routine use. The method is validated following the analytical performance parameters proposed by International Conference on Harmonization (ICH) [14].

MATERIALS AND METHODS:

Chemicals and Reagents: Imatinib working standard, impurity A, impurity B, impurity C and Imatinibmesylate tables100mg and 400 mg Imatinibmesylate tables 100 mg and 400 mg samples were provide by Intas Pharmaceutical Limited-Astron Division, Gujarat, India. Active pharmaceutical ingredient of Imatinib working standard, impurity A, impurity B and impurity C was with purity of 98.9%, 99.7%, 99.7% and 98.2% respectively. Tablet formulations containing 100 mg and 400 mg of Imatinib were prepared in laboratory as a process of developing finished product. HPLC grade acetonitrile and methanol (Merck, India), HPLC grade Triethylamine (Spectrochem, India), AR grade Sodium dihydrogen phosphate (Merck, India) were used. High purity water was prepared by using Millipore Milli-Q plus water purification system. The 0.45µ nylon filter was used to filter the sample solution.

Instrumentations:

All analysis work performed on Agilent HPLC 1100/1200 series equipped with DAD detector, consisting of quaternary pump and auto sampler. The output signal was monitored and processed by Chromeleon software.

Calibrated electronic single pan balance Mettler Toledo (Model: MX5, BT 224 S and CPA225D), pH meter of lab India (Model: Pico^{+}) and ultra sonic cleaner of equitron were also used during the analysis.

Chromatographic parameters:

The chromatographic column used was Eclipse XDB-C18 (150 mm × 4.6 mm) column with 5µm particles size of Agilent technologies make. The flow rate was maintained at 1.0 ml/minute. The detection wavelength of the method was 265 nm. The column oven temperature was maintained at 50°C and sample temperature was maintained at room temperature. The injection volume was 20 µl. The run time of standard and sample was 10 minute.

Diluent:

Prepare a mixture of 450 ml volumes of water and 550 ml volumes of methanol and mix well.

Mobile phase:

Prepare a mixture of 450 ml volumes of buffer preparation (weigh accurately about 1.5 g of sodium dihydrogen phosphate in to 500 ml of water and adjust the pH of the solution to 8.00 with Triethylamine) and 550 ml volumes of solvent mixture (prepared a mixture of 300 ml volumes of methanol and 200 ml volumes of acetonitrile). Filter through 0.45 µ nylon filter and degas before use.

Standard preparation:

Standard preparation was prepared by dissolve Imatinib working standard in diluent to obtain the concentration of 25 µg/ml of imatinib. Further final dilution was prepared with mobile phase.

Sample preparation:

Transfer an accurately weigh quantity of 5 intact tablets in to 250 ml volumetric flask. Add 150 ml of diluent and disperse for about 15 minutes and sonicate for 15 minutes taking care to maintain temperature of ultrasonic bath below 20°C. Dissolve and dilute to volume with diluent and mix well. Centrifuge the solution at 1500 rpm for 10 minutes and then filter through 0.45µ nylon filter. Discard first few ml of the filtrate. Transfer an accurately 5.0 ml of this solution into a 20 ml volumetric flask and dilute to volume with diluent and mix well. Further transfer 5.0 ml of this solution into a 100 ml volumetric flask and dilute to volume with mobile phase and mix well. Concentration of sample preparation was 25µg/ml.

Method validation:
The developed method was validated according to the current ICH guidelines for perform all parameters like specificity, stability of analyte in solution, system precision, method precision, intermediate precision, linearity, accuracy and robustness. Validation revealed the method is specific, rapid, accurate, precise, reliable and reproducible. Analytical method validation covers all acceptance criteria defined in ICH guideline. Formulation of Imatinib mesylate tablets 100 mg and 400 mg are step up and step down formulation.

Specificity:
Prepare single set of placebo preparation (as per sample preparation method) containing all inactive ingredients in the same proportion as in the formulation. Inject single injection of diluent and placebo preparation into the liquid chromatography 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. Impurity A, impurity B, impurity C preparation for identification were prepared by dissolve all impurities in diluent to obtain the concentration of 2 µg/ml of all impurities and final dilution was prepared with mobile phase. Prepare sample preparation as per method. Inject single injection of impurity A, impurity B, impurity C preparation for identification and duplicate injection of sample preparation in to liquid chromatographic system & record the chromatograms. Identify the peaks due to these all impurities and Imatinib by retention time. Prepare sample preparation spiked with known impurities sample (as per mentioned below). Inject duplicate injection of this solution in to the liquid chromatographic system and record the chromatogram and spectra between 200-400 nm. Demonstrate spectral homogeneity of the Imatinib peak.

Sample preparation spiked with known impurities: Transfer 5.0 ml of this sample preparation into a separate 100 ml volumetric flask and add 5.0 ml each of impurity A stock preparation, impurity B stock preparation and impurity C stock preparation into the volumetric flask. Dilute to volume with mobile phase and mix well. Concentration of all impurities stock preparation were 40µg/ml.

Blank, placebo, Imatinib standard and sample of tablets were exposed to Acid hydrolysis (4 mL of 5 M HCl, 60°C, 24 hours), Alkali hydrolysis (4 mL of 2 M NaOH methanolic, 60°C, 4 hours), Peroxide Oxidation degradation (4 mL of 3% H_2O_2, 90 minutes at room temperature), Thermal degradation (105°C in oven, 72 hours), Positive UV light degradation (sample exposed under UV light for 72 hours), Negative UV light degradation (sample exposed under UV light with cover the quartz tube to ensure non-permeation of UV light for 72 hours), Water hydrolysis (4 mL of water, 60°C, 72 hours) and Control stress (sample was kept in water bath for 72 hours at 60°C) degradation conditions. All force degradation conditions sample preparations were analyzed by proposed method on photodiode array detector.

Stability of analyte in solution:
Stability of analyte in solution for standard preparation and sample preparation will be performed at 23-27°C in not protected from light. Prepare the standard preparation and sample preparation as per method. Store above prepared solution under 23-27°C in not protected from light in tight flask. Inject duplicate injection of the stored solution at different time intervals at about initial, 6 hours, 12 hours, 18 hours, 24 hours and record the peak response at each time interval. Calculate the percentage difference in area of standard preparation and sample preparation at different time interval in comparison to initial respective area.

Linearity:
Linearity stock preparation was prepared by dissolve Imatinib working standard in diluent to obtain the concentration of 500µg/ml of linearity stock preparation and 100% linearity level considering standard preparation concentration 25.000 µg/ml. Prepared different linearity level solutions from linearity stock preparation in mobile phase with final concentrations from 20.000µg/ml, 22.500µg/ml, 25.000µg/ml, 27.500µg/ml and 30.000µg/ml of working concentration. Inject all the linearity solutions in duplicate 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, R2 value and %Y-intercept bias at 100%. Also calculate the relative standard deviation of response factor for each concentration level.

Precision:

System precision (system suitability):

Prepare a standard preparation as per method. Inject single injection of diluent and six replicate injections of the standard preparation into the liquid chromatographic system and record the chromatograms. Check the system suitability as per method. System suitability parameters and acceptance criteria limit are given in **Table 6**.

Method precision (Repeatability):

Prepare six sets of sample preparation as per method. Calculate the mean and relative standard deviation of test results.

Intermediate Precision:

Intermediate precision was studied on different day, different analyst and using different instrument, different column (same dimension, same supplier with different serial number). Calculate the mean and relative standard deviation of results. Compare the results obtained between method precision and intermediate precision study. Calculate percentage difference in results between method precision and intermediate precision study. Calculate the similarity factor f_2 value between the six results obtained between method precision and intermediate precision study.

Accuracy:

Prepare accuracy samples in triplicate preparation at three different concentration level 80%, 100% and 120% of the target concentration 25.000 µg/ml considering 100% accuracy level. An accurately weighed quantity of Imatinibmesylate drug substance and placebo were taken as per below mentioned accuracy **Table 1**. Perform accuracy sample preparation as per method. Calculate percentage accuracy of Imatinib for all accuracy samples. Calculate the mean percentage accuracy, relative standard deviation for each level. Also calculate the overall percentage accuracy and relative standard deviation for all this results.

TABLE 1: ACCURACY

Accuracy level	Weight of placebo in mg	Weight of Imatinib mesylate in mg	Concentration of Imatinib in µg/mL
80%	497.2	477.8	20.00
100%	377.7	597.3	25.00
120%	258.2	716.8	30.00

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usages. The small change in chromatographic condition were studies by testing effect of minor variation in column oven temperature (±2°C) (48°C and 52°C), variation in the flow rate (±0.2 ml/minute) (0.8 ml/minute and 1.2 ml/minute), variations in pH of mobile phase buffer (±0.2 pH) (7.8 pH and 8.2 pH), variation in extraction time (± 5 minute) (i.e. 10 minute and 20 minute) and variation in mobile phase composition (± 5%) (i.e. ±5% of solvent mixture). Calculate the percentage difference between the average results obtained by the varied method and method precision results. If system suitability is not comparable with unaltered method, perform the robustness with less variable parameters.

RESULTS AND DISCUSSION:

Method development and optimization of chromatographic parameters:

The method was started to develop from proper selection of stationary phase which depends upon the molecular weight, structure and solubility. Reverse phase technique was used to elute Imatinib peak. Non polar stationary phase (i.e. C8 and C18 column) and polar mobile phase was helpful in RP-HPLC method. Devlosil HG-5 (150mm X 4.6mm) 5µ at flow rate 1.0 ml/minute and column oven

temperature 30°C was used with mobile phase ratio 450 ml of solvent mixture [mixture of methanol and acetonitrile in the ratio of (300: 200) : buffer preparation [1.65 gm of sodium hydrogen phosphate into 550 mL of water and adjust pH 8.00 with Triethylamine. Filter through 0.45µ filter and degassed before use]. For better peak shape and reduce run time change the mobile phase ratio 550 ml of solvent mixture [mixture of methanol and acetonitrile in the ratio of (300:200)] : 450 ml of buffer preparation [1.5 gm of sodium hydrogen phosphate into 500 ml of water and adjust pH 8.00 with Triethylamine] and column oven temperature 50°C. Concentration of Imatinib was also reduce to decrease column loading. Eclipse XDB C18 column has wide pH range and good column life. Hence, Devlosil HG-5 column was replaced by Eclipse XDB C18. The maxima of Imatinib peak observed at 265 nm in **Fig. 5**. So, this wavelength was consider for determination of Imatinib in Imatinib mesylate tablets dosage form. And optimized so, minimize the area of test preparation due to decrease column load and increase column oven temperature to better peak shape and replace higher carbon loading stationary phase i.e. Eclipse XDB C18 column of (150mm X 4.6 mm) 5µ.

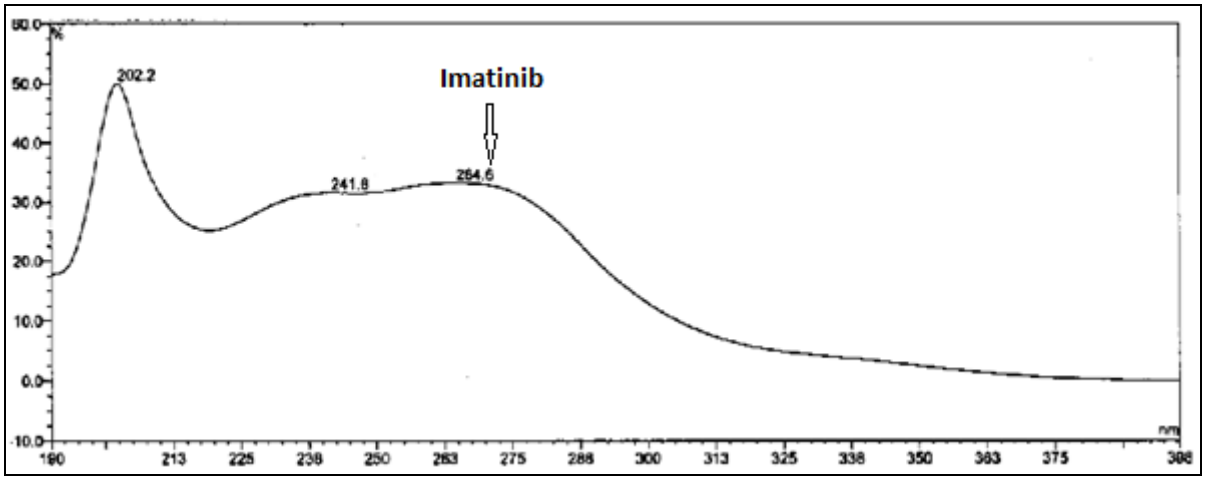


FIG. 5: HPLC CHROMATOGRAM OF IMATINIB STANDARD PREPARATION IN PDA FOR SELECTION OF WAVELENGTH

Results for method validation parameters:

Specificity:

No interference is observed at the retention time of Imatinib peak due to the diluent and placebo preparation. Impurity a peak is observed at the retention time of 1.277 minute in impurity a preparation for identification. Impurity B peak is observed at the retention time of 2.478 minute in impurity B preparation for identification. Impurity C peak is observed at the retention time of 7.495 minute in impurity C preparation for identification. Imatinib peak is observed at the retention time of 5.358 minute in standard preparation. Imatinib peak is observed at the retention time of 5.350 minute in sample preparation.

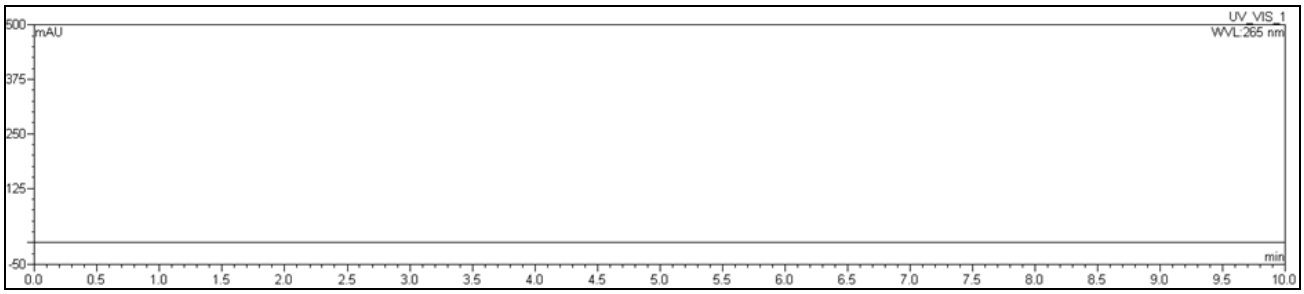
Impurity A, impurity B, impurity C and Imatinib peak observed at the retention time of 1.276, 2.479, 7.494 and 5.355 minutes respectively in sample preparation spiked with known impurities. Imatinib peak is well separated from known impurities peaks. Imatinib peak in the sample preparation spiked with known impurities is spectrally pure. Peak purity match value is 999.174 for sample preparation spiked with known impurities. Chromatograms of specificity are presented in **Fig. 6**.

Imatinib assay was dropped to 85.0%, 85.4%, 90.6%, 97.1%, 98.1%, 98.3%, 97.0% and 97.8% in acid hydrolysis, alkali hydrolysis, peroxide oxidation degradation, thermal degradation, positive UV light degradation, negative UV light degradation, water hydrolysis and control stress. Peak purity of Imatinib peak was found greater than 990, which indicates all peaks are well separate from Imatinib peak. Hence, there are no any interference. Degradation peaks were observed in chromatogram. Chromatographs of controlled sample, acid hydrolysis, alkali hydrolysis, peroxide oxidation degradation, thermal degradation, positive UV light degradation, negative UV light degradation, water hydrolysis and control stress degraded tablet samples are presented in

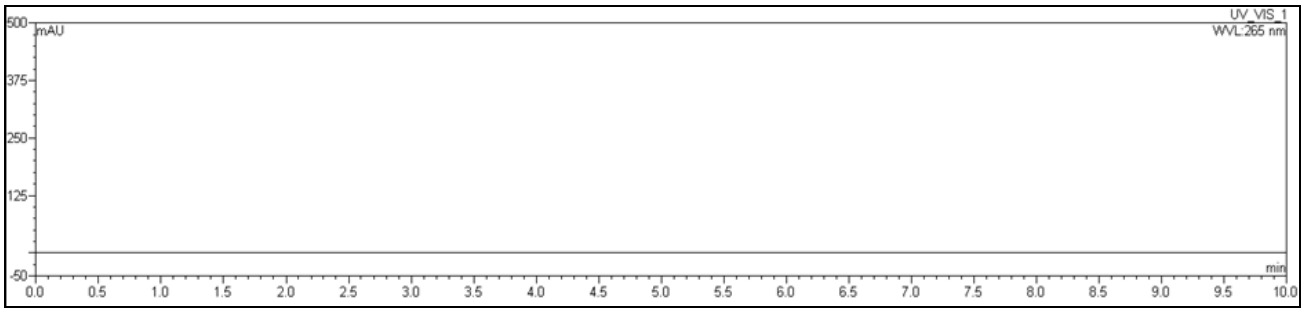
respectively. Chromatograms of force degradation study were presented in **Fig.6**. The results of force degradation study data are given in **Table 2**.

TABLE 2: FORCE DEGRADATION STUDY DATA

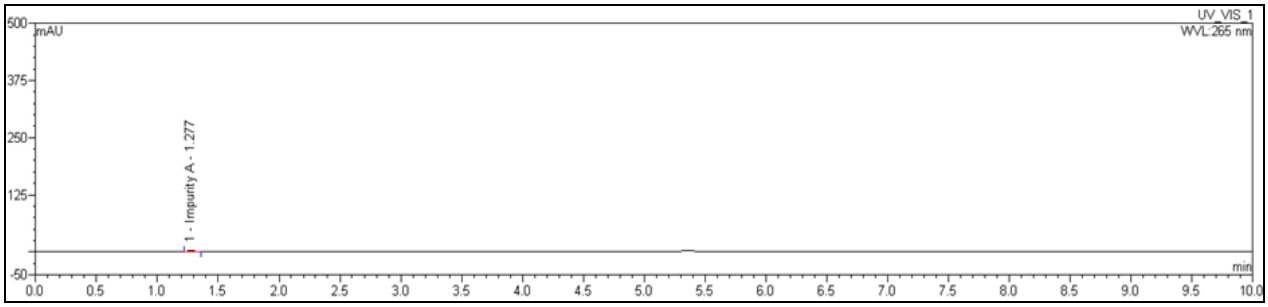
Degradation condition	% Assay of Imatinib
controlled sample (as such sample)	98.5%
Acid hydrolysis (4 mL of 5 M HCl, 60°C, 24 hours)	85.0%
Alkali hydrolysis (4 mL of 2 M NaOH methanolic, 60°C, 4 hours)	85.4%
Peroxide Oxidation degradation (4 mL of 3% H2O2, 90 minutes at room temperature)	90.6%
Thermal degradation (105°C in oven, 72 hours)	97.1%
Positive UV light degradation (sample exposed under UV light for 72 hours)	98.1%
Negative UV light degradation (sample exposed under UV light with cover the quartz tube to ensure non-permeation of UV light for 72hours)	98.3%
Water hydrolysis (4 mL of water, 60°C, 72 hours)	97.0%
Control stress (sample was kept in water bath for 72 Hours at 60°C)	97.8%



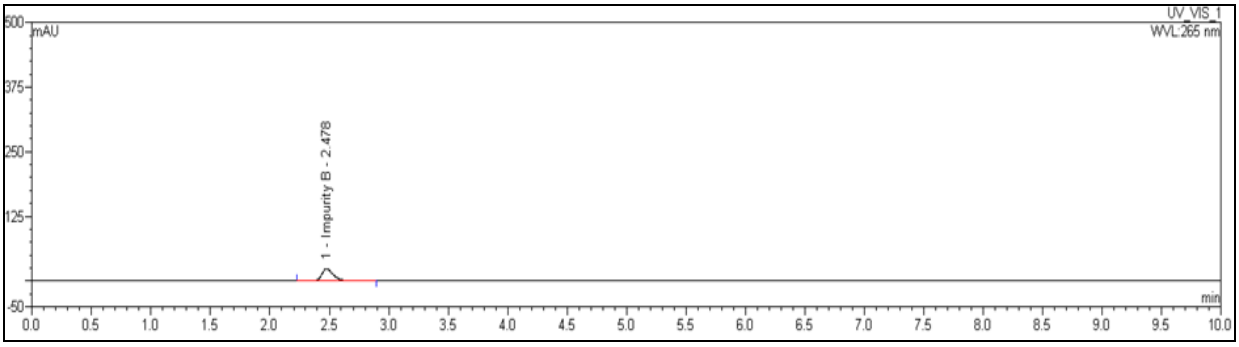
(A)



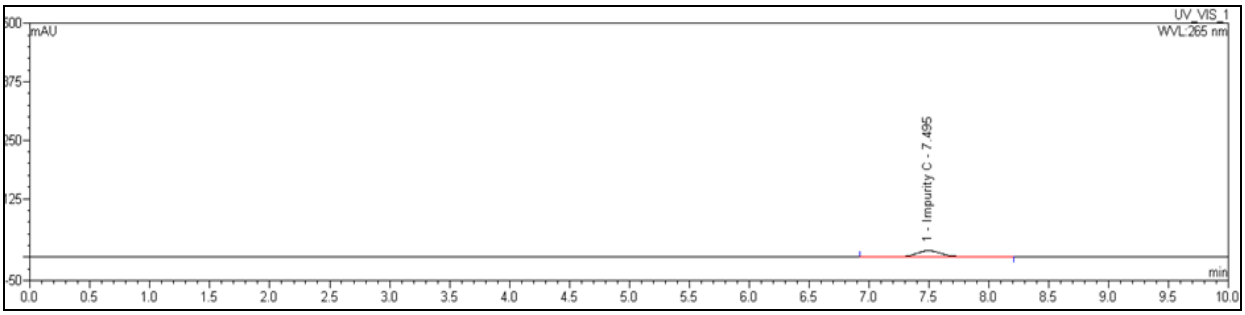
(B)



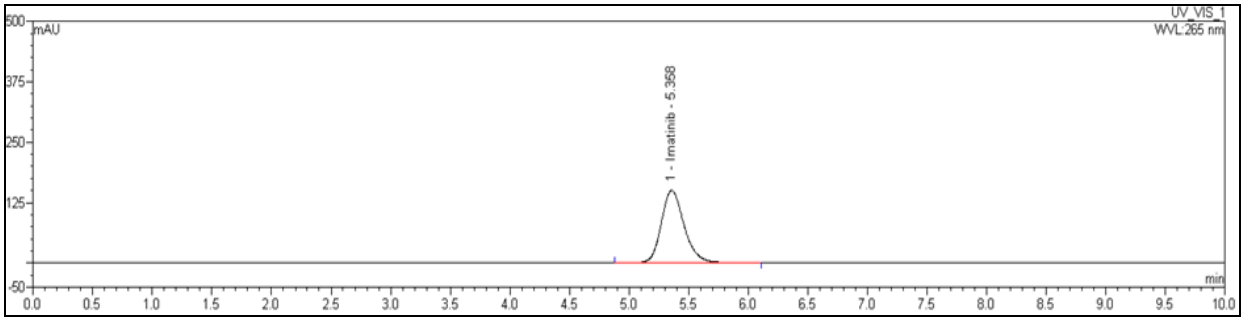
(C)


UV_VIS_1
WVL:265 nm
mAU
1 - Impurity B - 2.478
min

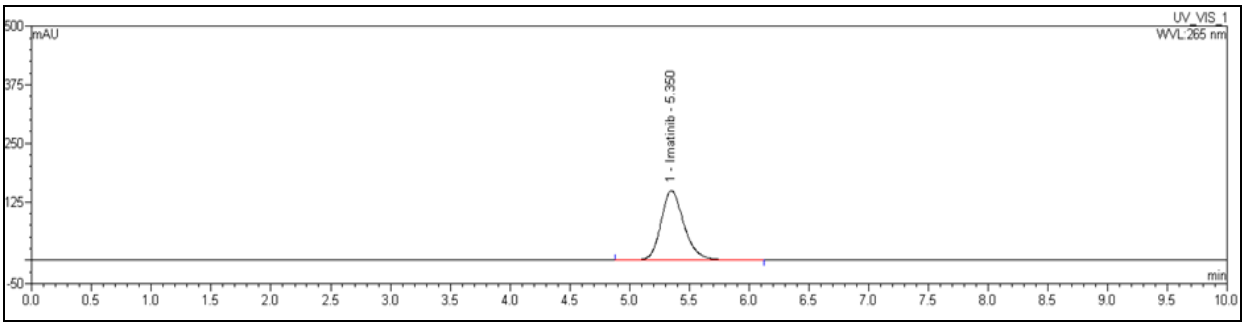
(D)


UV_VIS_1
WVL:265 nm
mAU
1 - Impurity C - 7.495
min

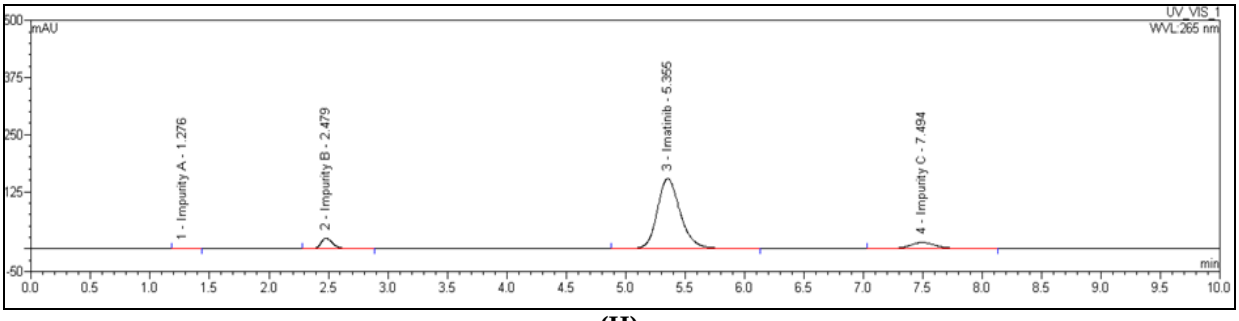
(E)


UV_VIS_1
WVL:265 nm
mAU
1 - Imatinib - 5.358
min

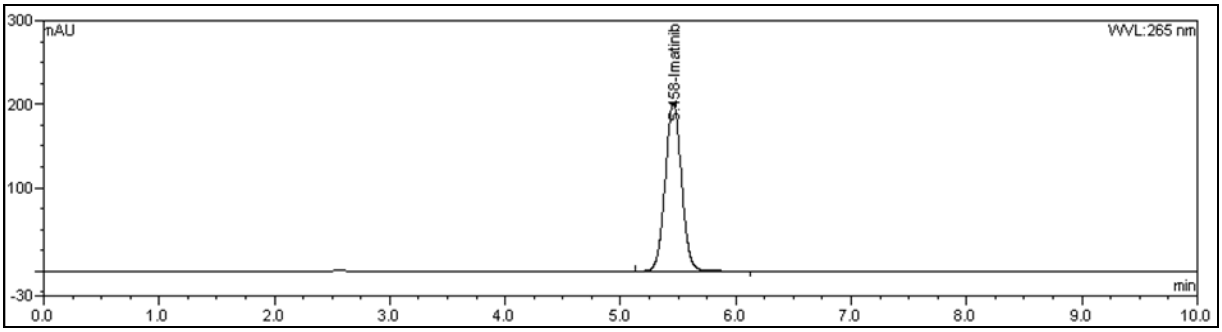
(F)


UV_VIS_1
WVL:265 nm
mAU
1 - Imatinib - 5.350
min

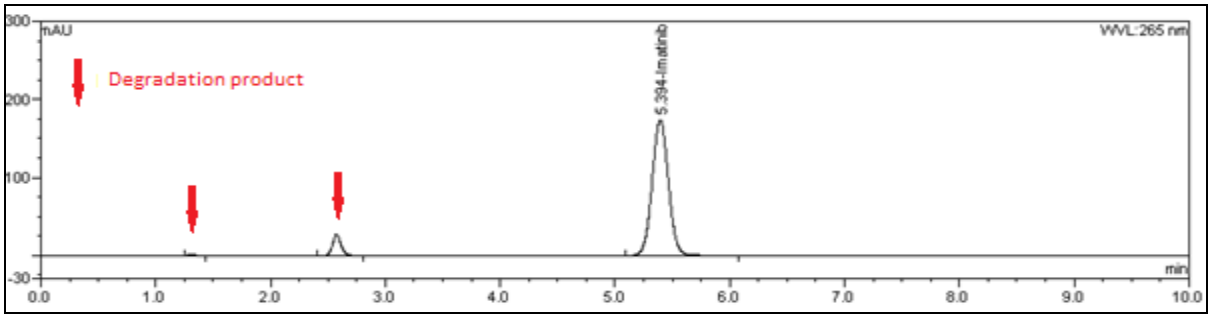
(G)


UV_VIS_1
WVL:265 nm
mAU
1 - Impurity A - 1.276
2 - Impurity B - 2.479
3 - Imatinib - 5.355
4 - Impurity C - 7.494
min

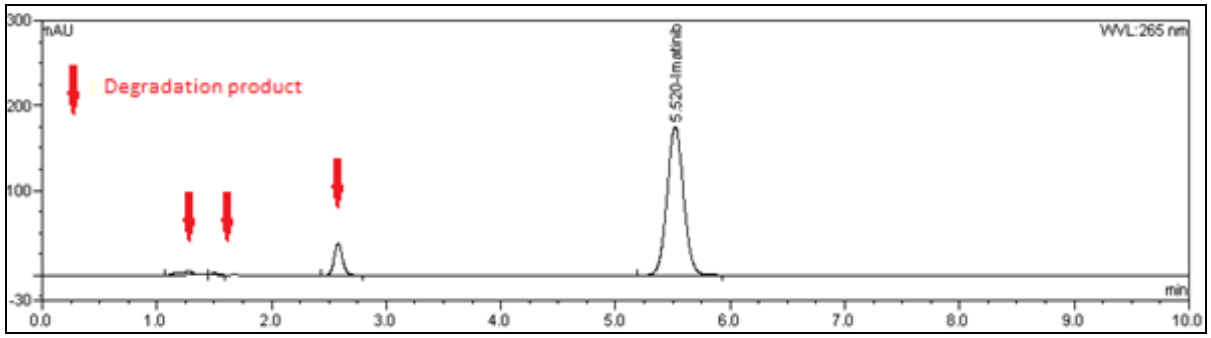
(H)


nAU
WVL:265 nm
5.458-Imatinib
min

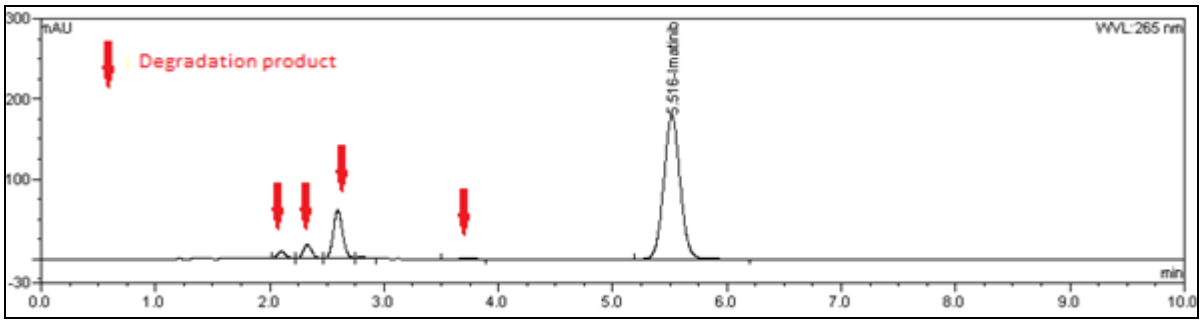
(I)


nAU
WVL:265 nm
Degradation product
5.394-Imatinib
min

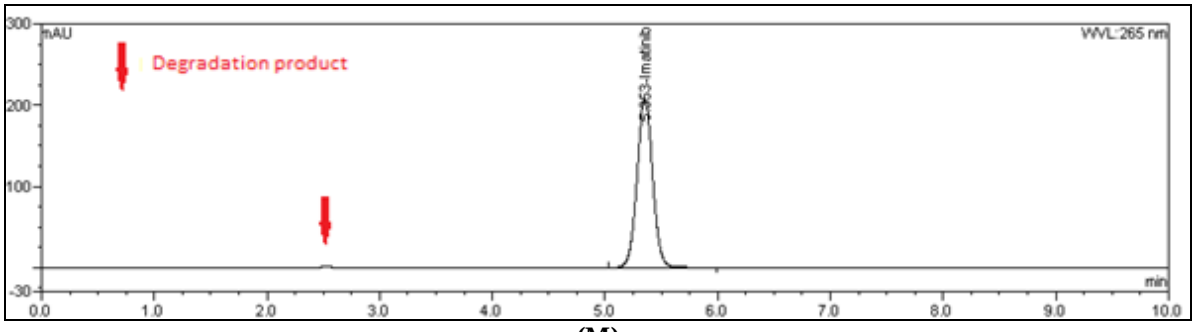
(J)


nAU
WVL:265 nm
Degradation product
5.520-Imatinib
min

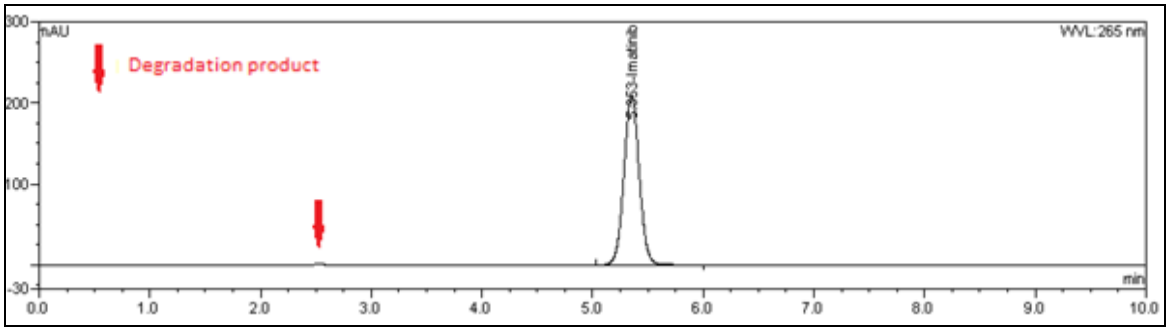
(K)


nAU
WVL:265 nm
Degradation product
5.516-Imatinib
min

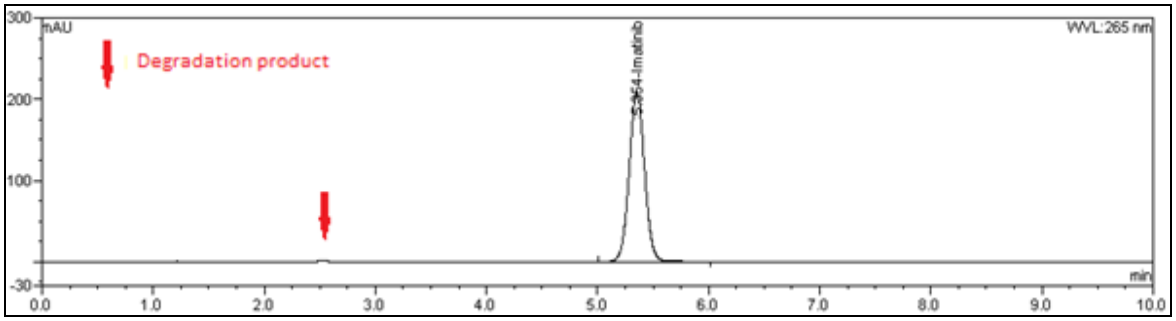
(L)


nAU
WVL:265 nm
Degradation product
5.363-Imatinib
min

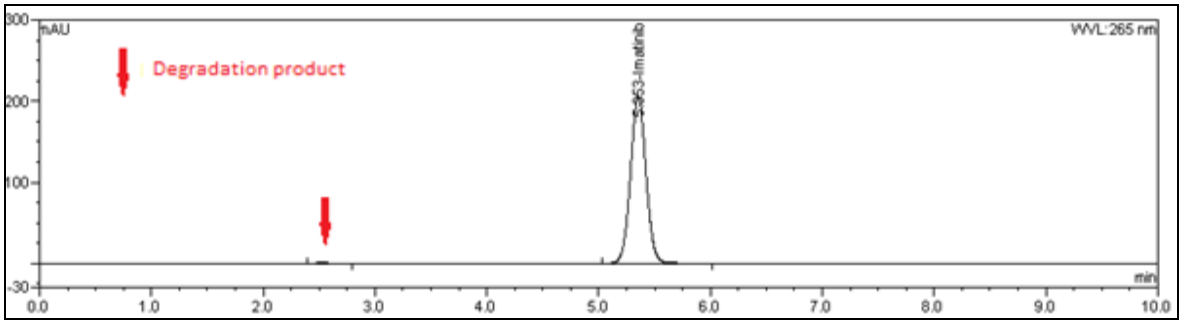
(M)



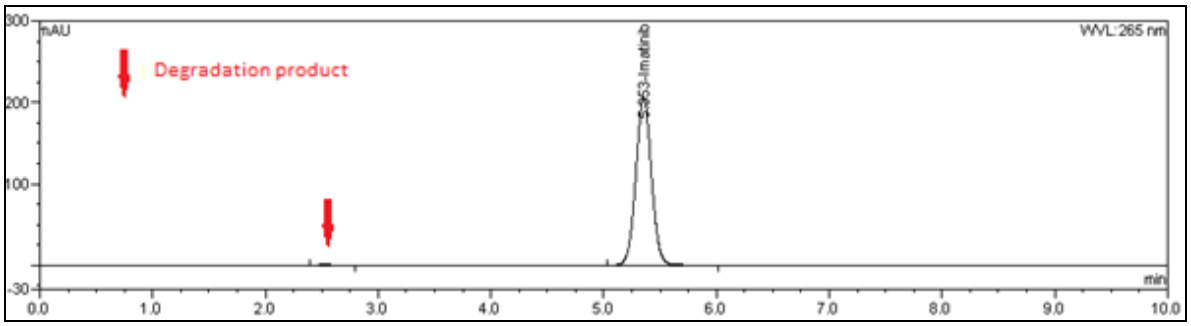
(N)



(O)



(P)



(Q)

FIG.6: CHROMATOGRAMS FOR SPECIFICITY

(A) Diluent (B) Placebo preparation (C) Impurity A preparation for identification (D) Impurity B preparation for identification (E) Impurity C preparation for identification (F) Standard preparation (G) Sample preparation (H) Sample preparation spiked with known impurities (I) Controlled sample (as such sample) (J) Acid hydrolysis (K) Alkali hydrolysis (L) Peroxide oxidation degradation (M) Thermal degradation (N) Positive UV light degradation (O) Negative UV light degradation (P) water hydrolysis (Q) Control stress.

Stability of analyte in solution:

Percentage difference of area of Imatinib peak is well within the limit for standard preparation and sample preparation up to 24 hours at 23-27°C in not protected from light. Hence, the stability of analyte in solution is stable for standard preparation and sample preparation up to 24 hours at 23-27°C in not protected from light. The results of stability

of analyte in solution study data are given in **Table 3**.

TABLE 3: STABILITY OF ANALYTE IN SOLUTION

Time interval	Standard preparation		Sample preparation	
	Area	%Difference	Area	%Difference
Initial	2022.9328	NA	2011.0842	NA
6 hours	2012.9349	0.49	2009.7440	0.07
12 hours	2024.8792	0.10	2013.2144	0.11
18 hours	2028.1749	0.26	2015.1087	0.20
24 hours	2029.4900	0.32	2016.6807	0.28

Linearity:

The correlation coefficient is 0.99990. Percentage relative standard deviation of response factor is 0.29. Percentage Y-intercept bias at 100% level is 1.5. Linearity curve for Imatinib is presented in **Fig. 7**. The results of linearity are given in **Table 4**.

TABLE 4: LINEARITY

% Linearity levels	Concentration of Imatinib in µg/mL	Mean Area	Response Factor
80	19.815	1661.0219	83.826
90	22.292	1871.0294	83.933
100	24.769	2071.3438	83.626
110	27.246	2282.9906	83.792
120	29.722	2476.1697	83.311
	Correlation Coefficient:		0.99990
	R^2 value:		0.99981
	Y-Intercept:		30.18639
	Slope:		82.45554
	%RSD of response factor:		0.29
	% Y-Intercept bias at 100% level:		1.5

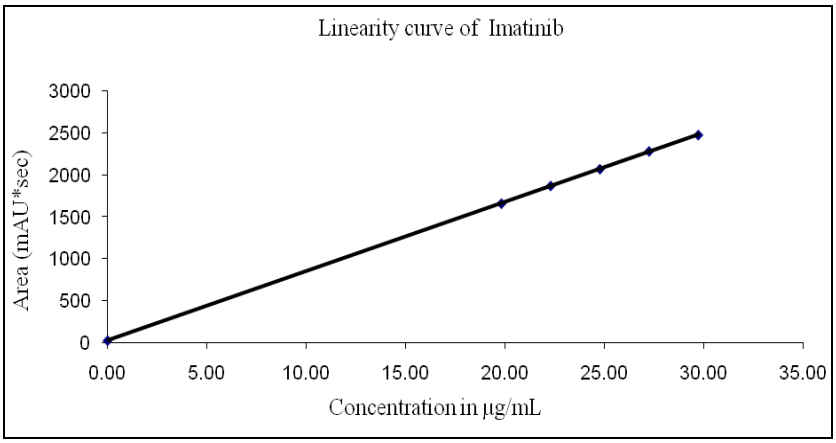


FIG. 7: LINEARITY CURVE FOR IMATINIB

Precision:

System Precision (System suitability): System suitability is well within the acceptance criteria. System suitability results are given in **Table 5**.

TABLE 5: SYSTEM PRECISION (SYSTEM SUITABILITY)

System suitability parameters	Results	Acceptance Criteria
In the chromatogram obtained with standard preparation,		
Relative standard deviation of replicate injections for Imatinib peak area	0.40	NMT 2.00%
Tailing factor for Imatinib peak	1.02	NMT 2.0
Theoretical plates of Imatinib peak	4500	NLT 1500
Mean retention time (in minute) of Imatinib peak	5.360	Not applicable
Difference in retention time (in minute) of Imatinib peak	0.0	NMT ±0.2 min. from average retention time

Method precision: Percentage relative standard deviation of six results is well within the limit. Results show good degree of precision. Method precision results are given in **Table 6**.

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. Intermediate precision results are given in **Table 6.**

TABLE 6: METHOD PRECISION AND INTERMEDIATE PRECISION

%Assay of Imatinib		
Set No.	**Method precision**	**Intermediate precision**
1	98.3	98.3
2	97.9	97.5
3	98.1	98.2
4	98.0	97.8
5	98.2	98.0
6	98.3	98.9
Mean	98.1	98.1
%RSD	0.17	0.49
%Difference		0.0
f_2 value		99.0

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. Accuracy results are given in **Table 7**.

TABLE 7: ACCURACY

Accuracy data for Imatinib				
Accuracy Level	**Set No.**	**Theoretical amount in µg/mL**	**Recovered amount in µg/mL**	**% Accuracy**
80%	Set-1	19.698	20.008	101.6
	Set-2	19.727	19.994	101.4
	Set-3	19.831	19.936	100.5
			Mean	101.2
			%RSD	0.58
100%	Set-1	24.810	24.781	99.9
	Set-2	24.632	24.781	100.6
	Set-3	24.740	24.807	100.3
			Mean	100.3
			%RSD	0.35
120%	Set-1	29.790	29.799	100.0
	Set-2	29.665	29.890	100.8
	Set-3	29.607	29.770	100.6
			Mean	100.5
			%RSD	0.41
Overall Mean:				100.6
Overall %RSD:				0.57

Robustness:

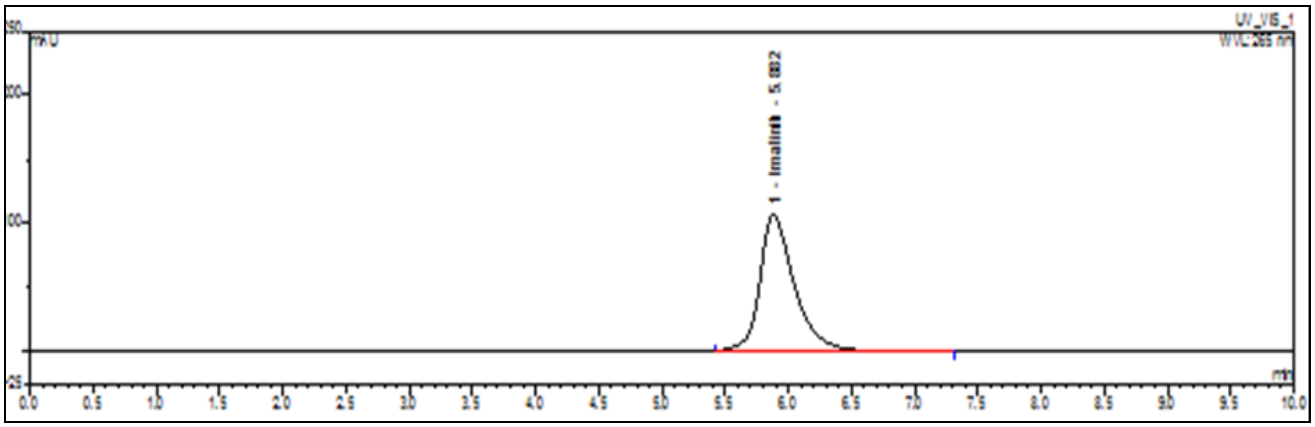
The robustness study was done by making small changes in chromatographic condition, pH of mobile phase and mobile phase composition in the optimized method. There was no considerable change in the peak areas and retention time. The parameters like percentage RSD of peak area, tailing factor and theoretical plates showed observance to the limit. The data of robustness are given in **Table 8** and **Table 9**. Specimen chromatograms for robustness parameters are given in **Fig.8**.

TABLE 8: COMPARISON OF SYSTEM SUITABILITY BETWEEN PRECISION AND ALTERED ROBUSTNESS PARAMETERS

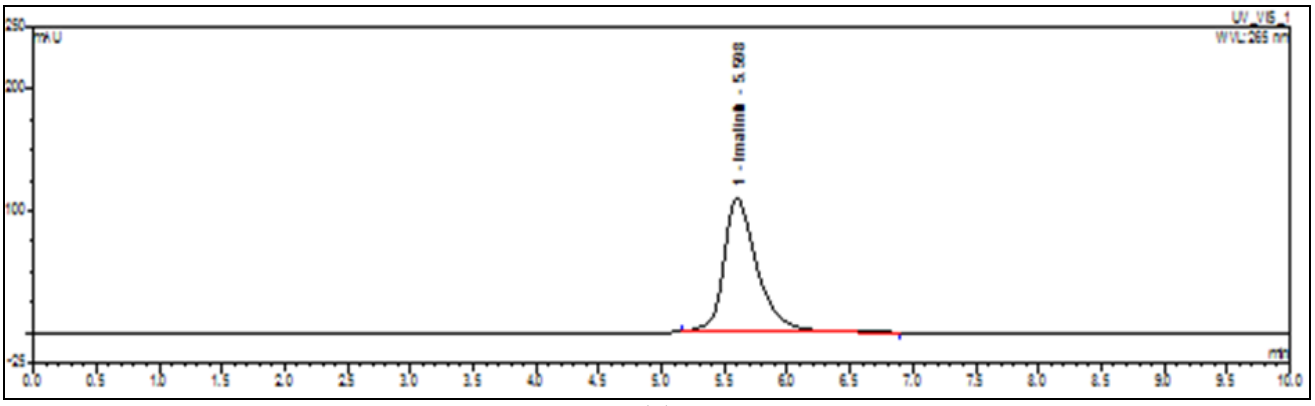
Robustness conditions	Mean RT (min)	Difference in RT (min)	%RSD of peak area	Tailing factor	Theoretical plates
System precision (Precision study)	5.360	0.0	0.40	1.02	4500
Column oven temperature: 48°C	5.882	0.0	0.14	1.30	2650
Column oven temperature: 52°C	5.598	0.1	0.10	1.35	2400
Flow rate: 0.8 mL/minute	7.239	0.1	0.27	1.42	2867
Flow rate: 1.2 mL/minute	4.784	0.0	0.21	1.35	2341
pH : 7.8 pH	5.470	0.0	0.16	0.95	6147
pH : 8.2 pH	5.403	0.0	0.18	0.98	6342
Mobile phase composition: -5% Solvent Mixture	6.698	0.1	0.25	0.84	6458
Mobile phase composition: +5% Solvent Mixture	4.399	0.0	0.19	0.85	5945

TABLE 9: COMPARISON OF RESULTS OF ASSAY PREPARATION FOR METHOD PRECISION AND ALTERED ROBUSTNESS PARAMETERS

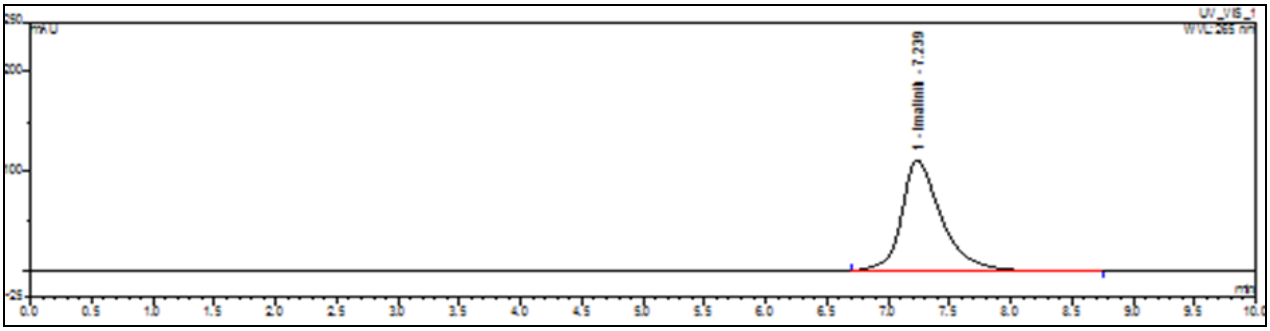
Robustness parameters	%Assay	% Difference
Method precision (mean results)	98.1	NA
pH : 7.8 pH	98.4	0.31
pH : 8.2 pH	98.5	0.41
Mobile phase composition: -5% solvent mixture	98.3	0.20
Mobile phase composition: +5% solvent mixture	98.9	0.82
Extraction time: 10 minute	98.5	0.41
Extraction time: 20 minute	98.8	0.71



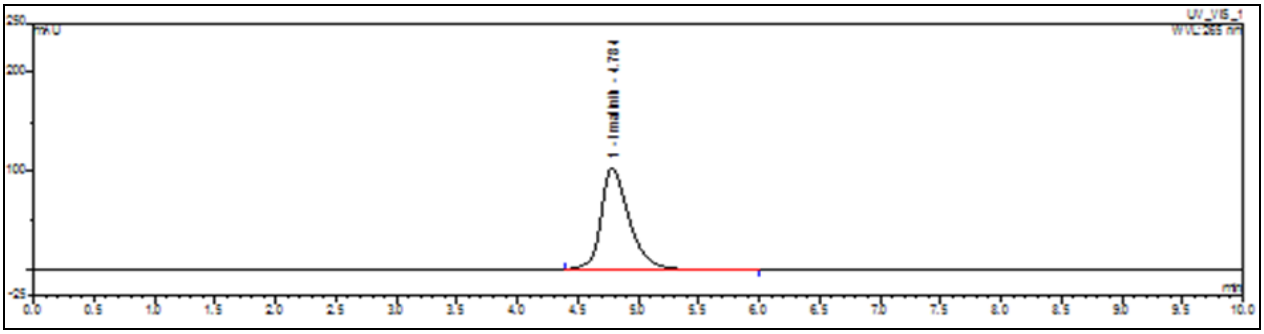
(A)



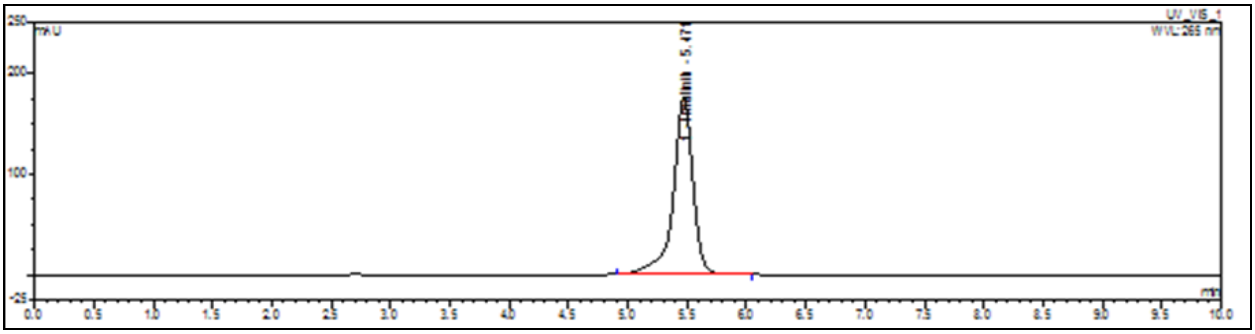
(B)


mAU
UV_VIS_1
WVL:265 nm
1 - Imalinib - 7.239
min

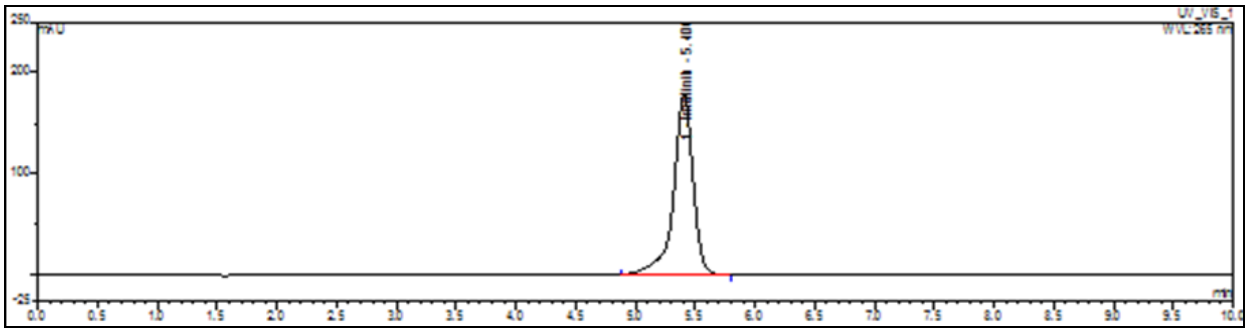
(C)


mAU
UV_VIS_1
WVL:265 nm
1 - Imatinib - 4.784
min

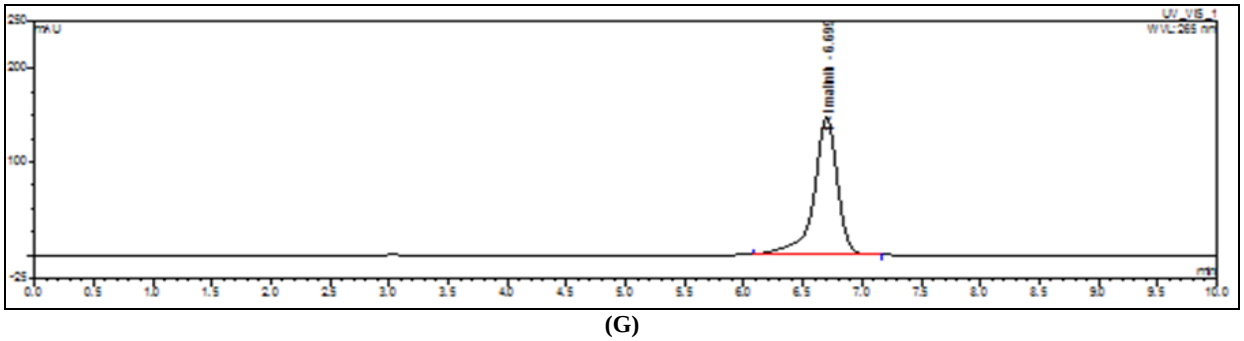
(D)


mAU
UV_VIS_1
WVL:265 nm
Imatinib - 5.471
min

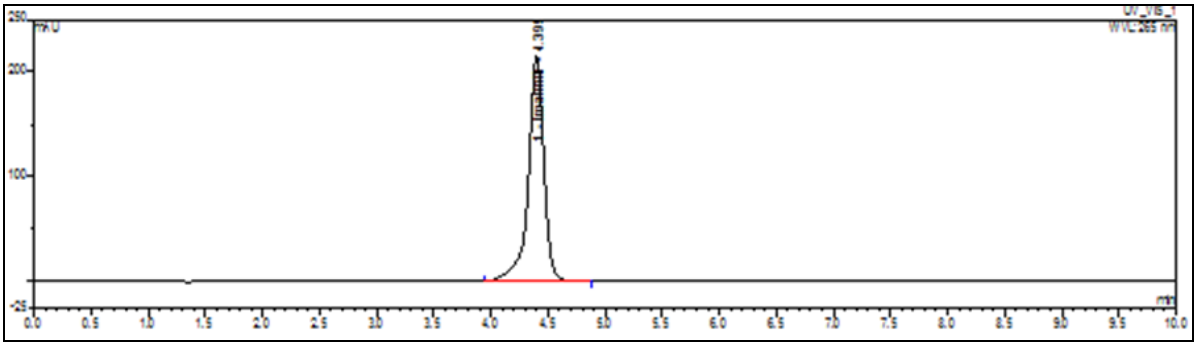
(E)


mAU
UV_VIS_1
WVL:265 nm
min

(F)


mAU
UV_VIS_1
WVL:265 nm
min

(G)



(H)

FIG.8: SPECIMEN CHROMATOGRAMS FOR ROBUSTNESS PARAMETERS

(A) Variation in column oven temperature (- 2°C): 48°C (Actual 50°C)
(B) Variation in column oven temperature (+ 2°C): 52°C (Actual 50°C)
(C) Variation in flow rate (- 0.2 mL/minute): 0.8 mL/minute (Actual 1.0 mL/minute)
(D) Variation in flow rate (+ 0.2 mL/minute): 1.2 mL/minute (Actual 1.0 mL/minute)
(E) Variation in pH of mobile phase buffer: (- 0.2 pH): 7.8 pH (Actual 8.0 pH)
(F) Variation in pH of mobile phase buffer: (+ 0.2 pH): 8.2 pH (Actual 8.0 pH)
(G) Variation in mobile phase composition (-5% solvent mixture)
(H) Variation in mobile phase composition (+5% solvent mixture)

CONCLUSION: An isocratic RP-HPLC assay method is successfully developed and validated for the determination of Imatinib in Imatinib mesylate tablet dosage form. The method is evaluated for all method validation parameters perform as per ICH guidelines. Method validation results has proven that the method is specific, rapid, precise, accurate, linear and robust. The method is stability-indicating and can be used for routine analysis in quality control and stability of samples. The run time of this method is relatively shorter 10 minutes with better solution stability for minimum 24 hours allows analysis of many samples per day in comparatively low cost.

ACKNOWLEDGEMENT: The authors are thankful to Intas Pharmaceutical Limited-Astron Division for providing all the necessary facilities to carry out this work.

AUTHOR'S STATEMENT: The author declare that we have no conflict of interest. Also, the article has not been published elsewhere and it has not been submitted simultaneously to any other publishers for the publication purposes.

REFERENCES:

1. Goldman JM and Melo JV: Chronic Myeloid Leukemia — Advances in Biology and New Approaches to Treatment. The New England journal of medicine 2003; 349(15):1451–1464.
2. Fausel C: Targeted chronic myeloid leukemia therapy: Seeking a cure. American journal of health system pharmacy 2007; 64(24 Suppl 15):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(5):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(3):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(9):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(3):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(2):722-726.
8. Velpandian T, Mathur R, Agarwal NK, Arora B, Kumar L and Gupta SK: Development & Validation of simple liquid chromatographic method with ultraviolet detection for the determination of Imatinib in Biological sample. Journal of Chromatogrphy. B Analytical technologies in the biomedical and life Sciences 2004; 804(2):431-434.
9. Vivekanand VV, Sreenivas RD, Vaidyanathan G, Sekhar NM, Avijit KS and Ramachandra PP: A Validated LC method for Imatinib Mesylate. Journal of pharmaceutical and biomedical analysis 2003; 33(5):879-889.
10. 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(2):14-18.
11. 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(3): 238-243.
12. Sandhya P, Vishnu PP and Anjali ND: Method development and validation of Imatinib Mesylate in Pharmaceutical dosage form by RP-HPLC. World Journal

of Pharmacy and Pharmaceutical Sciences 2013; 3(1):682-688.

13. 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(20):3283-3292.
14. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005.

How to cite this article:
Ayub M, Gul S, Afzal N and Eraj A: An effective clinical pharmacist communication may built patient trust on pharmacist over other healthcare providers: evidence based practice. Int J Pharm Sci Res 2015; 6(10): 4453-68.doi: 10.13040/IJPSR.0975-8232.6(10).4453-68.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)