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A stability-indicating RP-HPLC method development and validation for the related substances determination of Imatinib process impurities and their degradation products in tablet dosage form.

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Abstract: A sensitive, robust and selective stability indicating RP-HPLC method for the related substances determination of process impurities and their degradation products of Imatinib in tablet dosage form was developed and validated. Stability indicating power of the method was established by forced degradation experiments and mass balance study. The chromatographic separation was performed on Symmetry C18 (150 mm × 4.6 mm) 5µ make: Waters column, using gradient elution of mobile phase-A (prepare a mixture of 500 volumes of pH 3.0 buffer solution and 500 volumes of methanol) and mobile phase-B (prepare a mixture of 40 volumes of pH 3.0 buffer solution and 960 volumes of methanol) at a flow rate of 1.0 ml/minute. The buffer solution was prepared by dissolving 7.5 g of 1-octane sulfonic acid sodium salt in water and adjusting the pH to 3.0 with ortho-phosphoric acid. The column oven temperature and sample temperature was maintained at 27°C and ambient respectively. Detection was performed at 240 nm. The injection volume was set to 20µl and the run time of this method is 65 minutes. The retention time of the Imatinib peak was found to be about 21 minute. The method was further evaluated for its stability indicating capability by acid hydrolysis, alkali hydrolysis, water hydrolysis, oxidation degradation, thermal degradation and photolytic degradation. All acceptance criteria of International Conference on Harmonization guideline for validation were covered in method validation. This method can be used for quality control sample during manufacture and during stability sample analysis.
Keywords: Related substances, Imatinib, Stability-indicating, Development, Validation.

1. Introduction

Imatinib is an antineoplasticdrug used to treat leukemia, especially chronicmyelogenousleukemia (CML), certain types of adult acute lymphocytic leukemia and number of other cancers. It is also used in the treatment of gastrointestinal stromal tumors (GISTs) and a muscle cancer called dermatofibrosarcoma protuberans. Imatinib is chemically a 2-phenylaminopyrimidine derivative which functions as a specific inhibitor of a number of tyrosine kinase. Imatinib is like all tyrosine-kinase inhibitors acts by inhibiting a tyrosine kinase enzyme in this case BCR-Abl from phosphorylating subsequent proteins and starting the signalling cascade necessary for cancer growth and death. Thus, preventing the growth of cancer cells and

leading to their death by apoptosis. The BCR-Abl tyrosine kinase enzyme is over expressed in cancer cells only. Imatinib is one of the first anticancer drug to showthe potential targeted action [1-6].

Chemically Imatinib mesylate is 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamid methane sulfonate with molecular weight 589.7 g/mol. The chemical structure has shown in Figure 1. Imatinib mesylate is approved by the US food and drug administration for the treatment of chronic myeloid leukemia (CML) a rare kind of cancer. Imatinib is being sold under the trade name "Gleevec" or "Glivec" with dosage forms of 100mg and 400mg respectively. Imatinib has been also registered in Indian Pharmacopoeia (IP).

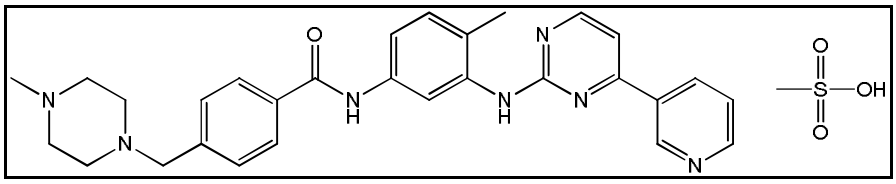


Figure 1: Imatinibmesylate

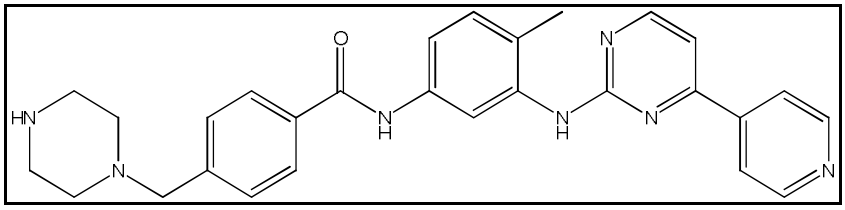


Figure 2: Impurity-A

One of the known process as well as degradation impurity-A of Imatinib is chemically 4-[(Piperizinyl)methyl]-N-[4-Methyl-3-[(4-pyridinyl)-2-pyrimidinyl amino] phenyl] benzamide as shown in Figure 2. There has been very few literature available related to various stability indicating analytical methods for quantification of Imatinib individually based on high performancethin layer chromatography (HPTLC) [7], high performance liquid chromatography (HPLC) [8-12].In present report we represent a robust and precise stability indicating RP-HPLC method developed for the estimation of degradation products as well as the known impurity-A of Imatinib. The newly developed method was successfully validated according to the ICH guidelines[13].The method has been proved to be specific, precise, accurate, linear, reproducible and robust.

Stability testing forms an important part of the process of drug development. The aim of stability testing is to provide evidence on how the quality of a drug varies with time under the influence of a variety of environmental factors such as temperature, humidity, which enables recommendation of storage conditions, retest periods and establishing shelf life. The content of related substances of Imatinib drug product is required to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines [14].This method is also proved to be stability-indicating because it can well separate all degradation peaks from the Imatinib peak that are present in stress degraded samples or in aged stability samples.Thus, this method can be used for quality control sample during manufacturing as well as during stability sample analysis.

2. Experimental

2.1. Chemicals and Reagents

Analytical reagent grade 1-octane sulfonic acid sodium salt (Spectrochem, India), HPLC grade methanol (Merck, India) and ortho-phosphoric acid (Spectrochem, India) were used in the study. Standard drug Imatinib was obtained as working standard from Intas Pharmaceutical Limited-Astron Division, Gujarat, India. Imatinib, known impurity-A and Imatinib mesylate tables 100mg and 400mg samples were provide from Intas Pharmaceutical Limited-Astron Division, Gujarat, India. Imatinib working standard and known impurity-A was with purity of 98.9%and 98.2% respectively. The nylon filters with pore size of 0.45µm(Advanced micro device) were used to filter solutions.

2.2. Instrumentations

HPLC system (Make: Agilent HPLC 1100/1200 series, USA) equipped withauto sampler and quaternary pump with degasser was used.The column component having temperature control, UVdetector and DAD detector was used during the analysis. Symmetry C18 (150 mm X 4.6 mm, 5µ) Make: Waters column was used.Chromatographic data were monitored and processed by using Chromeleon software.Calibrated analytical balance Sartorius (Model: BT 224S), Mettler Toledo (Model: MX5), pH meter of lab India (Model: Pico+) and ultrasonic cleaner of equitron were used during the analysis.

2.3. Chromatographic conditions

The chromatographic separation was carried out on a Symmetry C18 column (150 mm × 4.6 mm) 5µ of waters make. The separation was achieved by at flow rate of 1.0 ml/minute with a gradient program of (T/%B) 0.01/00; 15/20; 45/65; 55/65; 60/00; 65/00. The detection was observed at a wavelength of 240 nm. The column oven temperature was maintained at 27°C and sample temperature was maintained at ambient temperature. The injection volume was 20µl. The retention time of Imatinib peak was obtained about 21 minute. Prepare a mixture of 450 ml volumes of water and 550 ml volumes of methanol and mix well as diluent.

2.4. Preparation of buffer solution

Dissolved an accurately weighed 7.5g of 1-octane sulfonic acid sodium salt in to 1000 ml of water. Adjust the pH 3.00 with ortho-phosphoric acid. Filter through 0.45 µ nylon filter.

2.5. Preparation of Mobile phase-A

Prepare a mixture of 500 ml volumes of buffer solution and 500 ml volumes of methanol and mix well. Degas before use.

2.6. Preparation of Mobile phase-B

Prepare a mixture of 40 ml volumes of buffer solution and 960 ml volumes of methanol and mix well. Degas before use.

2.7. Preparation of solutions

2.7.1. Diluted standard preparation

Diluted standard preparation was prepared by dissolve Imatinib working standard in diluent to obtain the concentration of 1µg/ml of imatinib.

2.7.2. Sample preparation

Transfer an accurately weigh quantity of 5 intact tablets in to 250 ml volumetric flask. Add 150 ml of diluent, 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, mix well (concentration of sample stock preparation was 2000µg/ml). Centrifuge the solution at 1500 rpm for 10 minutes and then filter through 0.45µ nylon filter. Discard first few ml of the filtrate. Further, transfer an accurately 5.0 ml of this solution into a 20 ml volumetric flask and dilute to volume with diluent and mix well. Concentration of sample preparation was 500µg/ml.

2.8. Method validation

The proposed method was validated for the related substances of Imatinib by HPLC as per ICH guidelines for perform all parameters like specificity, stability of analyte in solution, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy and robustness. Validation shown the method is specific, accurate, precise, reliable and reproducible. Analytical method validation covers all acceptance criteria defined in ICH guidelines.

2.8.1. Specificity

Specificity studies were performed to show selectivity and stability indicating capacity of the proposed method. Specificity of the method was studied by injecting single injection of diluent, placebo preparation (as per sample preparation method) containing all inactive ingredients in the same proportion as in the formulation.

Known impurity-A preparation for identification was prepared by dissolve in methanol to obtain the concentration of 1µg/ml and final dilution was prepared with diluent. Prepare diluted standard preparation, sample preparation as per method and Sample preparation spiked with known impurity-A as per mentioned below.

Sample preparation spiked with known impurity-A: Transfer 5 ml of this sample stock preparation (concentration of 2000µg/ml) into 20 ml volumetric flask and add 1 ml known impurity-A stock preparation into the volumetric flask. Dilute to volume with diluent and mix well. Concentration of known impurity-A stock preparation was 20µg/ml.

Inject single injection of known impurity-A preparation for identification and injection of diluted standard preparation, sample preparation, sample preparation spiked with known impurity-A in to liquid chromatographic system & record the chromatograms. Identify the peaks due to these known impurity-A and Imatinib by retention time.

Force degradation studies were performed on Imatinib formulation to evaluate the stability indicating nature of the proposed method for the determination of assay(for mass balance only) and related substances of Imatinib mesylate in Imatinib mesylate tablet in the presence of excipients.Individual impurity, total impurities and degradation products were calculated by diluted standard preparation. Assay was calculated by area normalization for mass balance only. Blank as diluent, placebo, Imatinib diluted standard and sample of tablets were exposed to acid hydrolysis (Treat 5 intact tablets with 4.0 ml of 5M hydrochloric acid and keep undisturbed for 24 hours at 60°C temperature), Alkali hydrolysis (Treat 5 intact tablets with 4.0 ml of 2M sodium hydroxide methanolic and keep undisturbed for 4 hours at 60°C temperature), Peroxide oxidation degradation (Treat 5 intact tablets with4.0 ml of 3% hydrogen peroxide and keep undisturbed for 90 minutes at room temperature), Thermal degradation (Heat intact tablets for 72 hours at 105°C in oven), photolytic degradation (Expose the intact tablets under UV light for 72 hours) and water hydrolysis (Treat 5 intact tablets with 4.0 ml of water and keep undisturbed for 72 hours at 60°C temperature) degradation conditions.All force degradation conditions sample preparations were analyzed by proposed method on photodiode array detector.

2.8.2.Stability of analyte in solution

Stability of analyte in solution for diluted standard preparation prepared as per method and sample preparation spiked with known impurity-A prepared as per specificity have been performed at 23-27°C and not protected from light. Store above prepared solution under 23-27°C and not protected from light in tight flask. Inject single injection of the stored solution at different time intervals at about initial, 8, 16, 24 and 48 hours and record the peak response at each time interval. In diluted standard preparation, calculate the relative standard deviation of the initial and each time interval area individually. Establish the time interval up to which the standard is stable based on the relative standard deviation value. In sample preparation spiked with known impurity-A, determine the percentage impurity and calculate the absolute difference or percentage difference as applicable in results value at each time interval against respective initial results.

2.8.3. Limit of detection (LOD) & Limit of quantification (LOQ)

For LOD and LOQ determination, prepare five different concentration level linearity of known impurity-A and Imatinib ranging from 10% to 25% of limit concentration as per ICH guideline (limit concentration of known impurity-A and Imatinib 100% linearity level is 1µg/ml). Inject each standard solution and plot a linearity curve of area versus concentration. From the linearity data, find out the slope(S) and residual standard deviation (σ) of the regression line. Calculate the LOD and LOQ concentration from the equation are given 3.3*σ/S and 10*σ/S respectively.

For LOD and LOQ confirmation, prepare the LOD and LOQ solutions obtained from the above formula. The LOD and LOQ solutions containing both Imatinib and known impurity-A were injected six replicate injections to achieve LOD and LOQ by signal to noise ratio (S/N) method as per ICH guideline. The percentage relative standard deviation values were determined for each peak by injecting six replicates at LOQ concentration level.

2.8.4. Linearity

The linearity of the method was prepared different linearity level solutions in diluent with final concentrations from LOQ, 0.500, 0.700, 0.900, 1.000, 1.100 and 1.200µg/ml (LOQ to 120%) of limit concentration (1µg/ml) for Imatinib and known impurity-A.Plot a linearity curve of concentration in µg/ml verses area. Calculate the response factor for each concentration level. Calculate correlation coefficient, slope of regression line, Y-intercept, R^2value, %Y-intercept bias at 100% concentration level. Calculate relative standard deviation of response factor for 50% to 120% concentration level.

2.8.5. Precision

2.8.5.1. System precision

Prepare diluted standard preparation as per method. Inject six replicate injections of diluted standard preparation in to liquid chromatographic system and record the chromatograms. Determine the mean and relative standard deviation of replicate injections with respect of area and retention time for Imatinib peak. Record tailing factor and theoretical plates for the Imatinib peak.

2.8.5.2. Method precision

Prepare six sets of sample preparation spiked with known impurity-A prepare same as per specificity. Calculate the mean and relative standard deviation of test results.

2.8.5.3. Intermediate precision

To demonstrate the intermediate precision study, repeat the method precision study using same sample preparation but under different conditions like different day, different analyst, different instrument, different column (Same dimension, same supplier with different serial no. column).Calculate the mean and relative standard deviation of six sets test results. Compare the results obtained between method precision and intermediate precision study.Calculate absolute difference or percentage difference as applicable in impurity results value obtained between method precision and intermediate precision study.

2.6. Accuracy (Recovery)

To perform the accuracy of the proposed method, recovery experiments were carried out by standard addition technique. The accuracy of the method was calculated in triplicate preparation at four different concentration levels - LOQ, 50%, 100% and 120% of the limit concentration 1µg/ml considering 100% accuracy level.

To prepare recovery solution for known impurity-A, prepare sample preparation as such to be used for subtracting the known impurity-A result in the recovery solutions. Known impurity-A stock solution preparation was prepared by dissolve known impurity-A in methanol and make up with diluent to obtain the concentration of 20µg/ml. Prepare recovery solution for known impurity-A as per below mentioned Table 1.

Table 1: Recovery solution for Impurity-A

Recovery level	Weight of sample to be taken in mg	Impurity-A stock solution to be taken in ml	Recovery solution in diluent (Stock solution)	Stock solution taken in ml	Final volume with diluent in ml	Concentration of Impurity-A in µg/ml
LOQ	5 Intact tablet	**To be decided after LOQ confirmation**				
50%		25.0	250	5.0	20	0.500
100%		50.0	250	5.0	20	1.000
120%		60.0	250	5.0	20	1.200

Table 2: Recovery solution for unknown impurities

Recovery level	Weight of placebo to be taken in mg	Imatinib stock solution to be taken in ml	Recovery solution in diluent (Stock solution)	Stock solution taken in ml	Final volume with diluent in ml	Concentration of Imatinib in µg/ml
LOQ	377.5	To be decided after LOQ confirmation				
50%	377.5	1.0	250	5.0	20	0.500
100%	377.5	2.0	250	5.0	20	1.000
120%	377.5	2.4	250	5.0	20	1.200

To prepare recovery solution for unknown impurities spiked Imatinib working standard, into placebo. Transfer an accurately weigh quantity of placebo powder and required volume of Imatinib stock solution as per below mentioned Table 2. Imatinib stock solution preparation was prepared by dissolve Imatinib working standard in diluent to obtain the concentration of 500µg/ml.

Calculate the percentage recovery for known impurity-A and Imatinib for all recovery samples. Calculate the mean percentage recovery, relative standard deviation for each level. Also calculate the overall percentage recovery and relative standard deviation for all results.

2.7. Robustness

To determine the robustness of the analytical method, experimental conditions were deliberately altered. The small change in chromatographic condition were studied by testing influence of minor variation in the flow rate (±0.2 ml/minute) (0.8 ml/minute and 1.2 ml/minute), variation in column oven temperature (±2°C) (25°C and 29°C), variations in pH of mobile phase buffer (±0.2 pH) (2.8 pH and 3.2 pH), variation in extraction time (± 5 minute) (i.e. 10 minute and 20 minute), variation in mobile phase-A composition (± 5%) (i.e. ±5% of methanol solvent in mobile phase-A mixture) and variation in mobile phase-B composition (± 2%) (i.e. ±2% of methanol solvent in mobile phase-B mixture). Calculate the absolute difference or percentage differenceas applicable in individual impurity and total impurities results value obtained between the varied method and method precision study results value. If system suitability is not comparable with unaltered method, perform the robustness with less variable parameters.

3. Results and Discussion

3.1. Method development and optimization of chromatographic parameters

The method was started to develop at lower carbon loading Hypersil BDS C18 (150 mm × 4.6 mm) 5µ column with isocratic elution by using as mobile phase a mixture of contain 7.5 g/l potassium dihydrogen phosphate adjust pH 3.0 with ortho-phosphoric acid as buffer solution and methanol ratio of 50:50 (V/V) at a flow rate 1.0 ml/minute, column oven temperature was 27°C, detection was performed at 240 nm, sample concentration was 500µg/ml and 20µl injection volume was injected. But this method trail known impurity-A peak merged with imatinib peak. Then, higher carbon loading column may be helpful to well separate both peak. So, Hypersil BDS C18 (150 mm × 4.6 mm) 5µ column was replaced by Symmetry C18 (150 mm × 4.6 mm) 5µ column. But this trial in Symmetry C18 column both peak Imatinib and known impurity-A were not separate. Further, ion pairing reagent may be helpful for well separation peak. Hence, potassium dihydrogen phosphate reagent which was used in mobile phase as buffer solution replaced with 1-octane sulfonic acid sodium salt. But till peak shape of imatinib was not good. Peak of known impurity-A and Imatinib were merged with each other impurity. However, to further the gradient program was used with a gradient elution where the gradient program (T/%B) was set as 0.01/0; 15/20; 45/40; 55/65; 60/0; 65/0 at flow rate 1.0 ml/minute. In this gradient program, this method was introduced for separation of all impurities and Imatinib peak in which mobile phase-A contain 7.5 g/l of 1-Octane sulfonic acid sodium salt adjust pH 3.00 with ortho-phosphoric acid as buffer and methanol ratio 50:50 (V/V), While mobile phase-B Contain same buffer and Methanol ratio 4:96 (V/V) in with both peak Imatinib and known impurity-A are well separate but during force degradation study unknown peak are merged with known impurity-A peak.So, further the gradient program was changed with a gradient elution where the gradient program (T/%B) was set as 0.01/0; 15/20; 45/65; 55/65; 60/0; 65/0 at flow rate 1.0 ml/minute. Known impurity-A, Imatinib and unknown impurities peaks are well separated in this

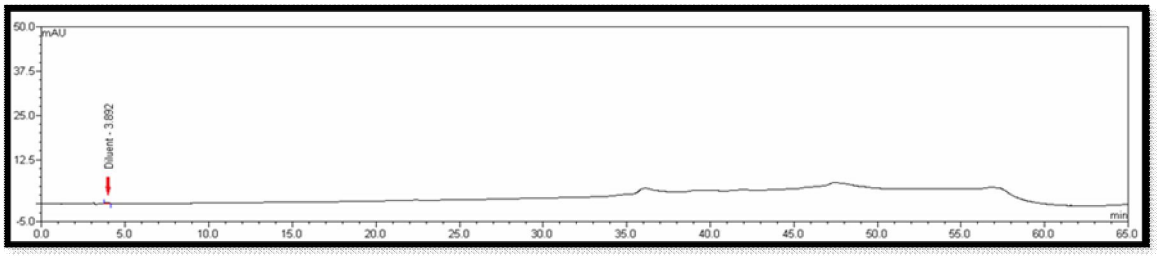
gradient program. Hence, force degradation study was conducted in this final program and finalized this method for further validation.

3.2. Method validation

3.2.1. Specificity

No interference is observed at the retention time of Imatinib and known impurity-A peak due to the diluent and placebo preparation. Peak obtained due to known impurity-A at the retention time of 17.602 minute inknown impurity-A solution for identification. Imatinib peak is observed at the retention time of 21.066 minute in diluted standard preparation. Peak obtained due to Imatinib and four unknown impurity at the retention time 20.966, 5.977, 11.201, 14.231 and 31.730 minutes respectively in sample preparation as such. Peak obtained due to known impurity-A, Imatinib and unknown impurity at the retention time of 17.730, 21.065 and 31.688 minutes respectively in sample preparation spiked with known impurity-A. Imatinib peak is well separated from known impurity-A and all unknown impurities peaks. Imatinib peak in the sample preparation spiked with known impurity-A is spectrally pure. Peak purity match value is 998 and 999 respectively known impurity-A and Imatinib for sample preparation spiked with known impurity-A. Chromatograms of specificity were presented in Figure 3.

(A)



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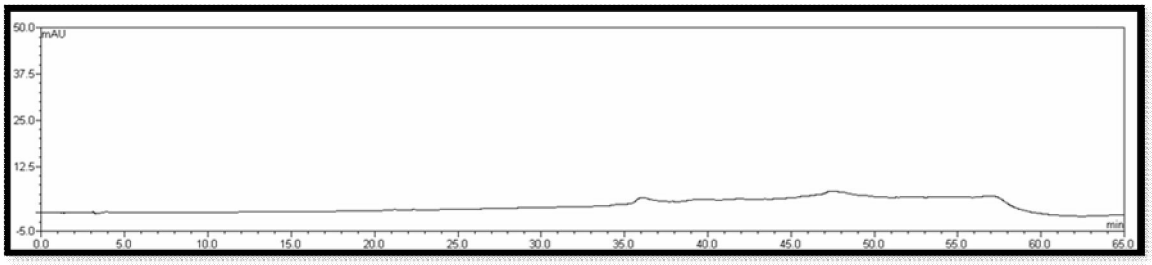

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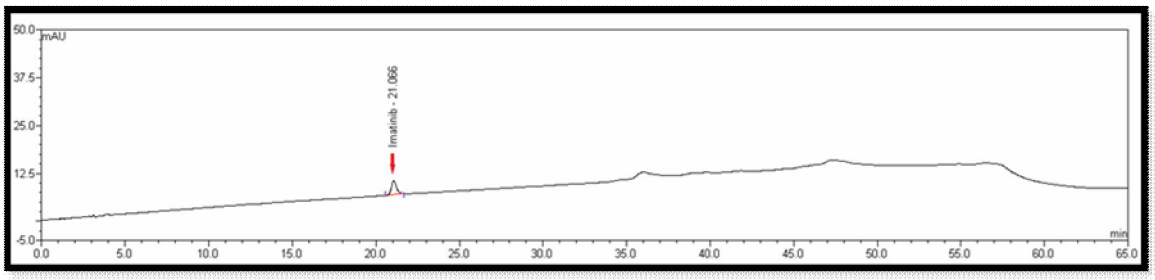

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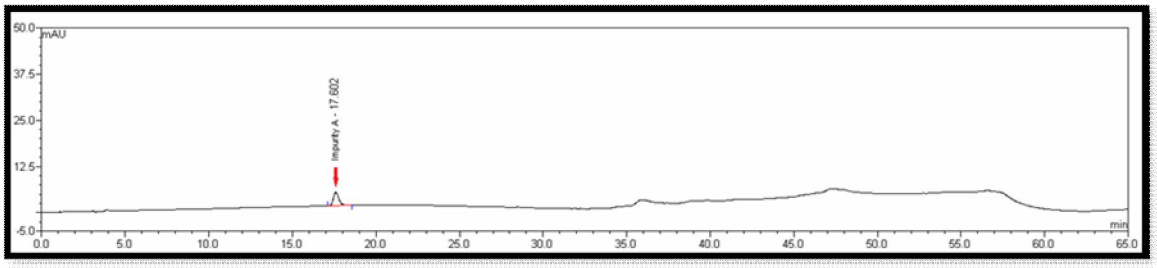
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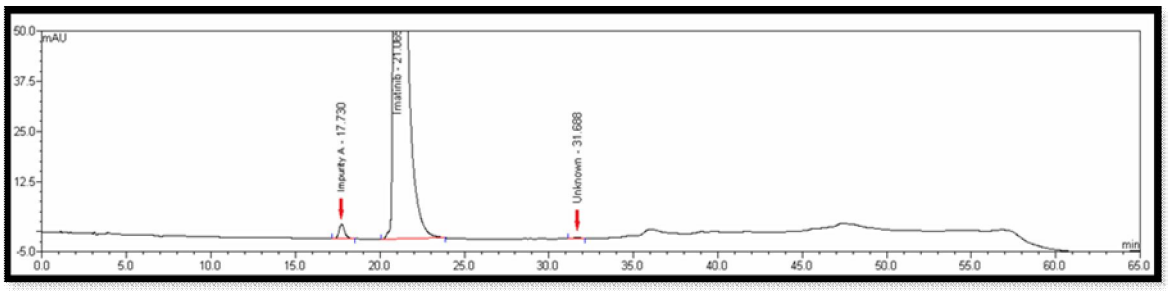
min

(E)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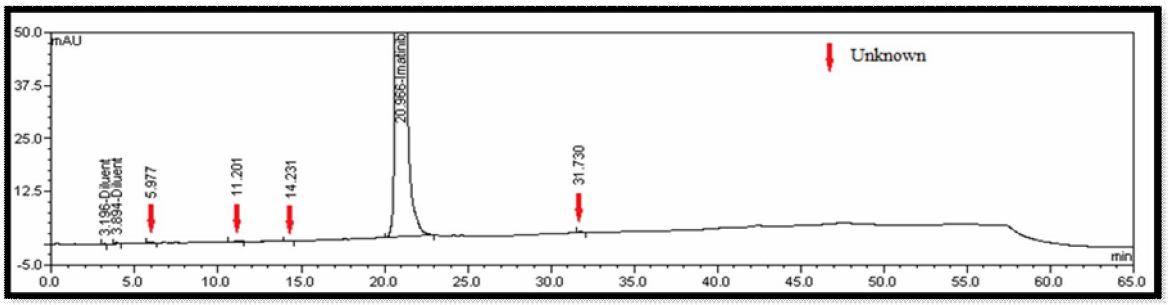
Imatinib - 21.083
Unknown - 31.688
min

(F)


mAU
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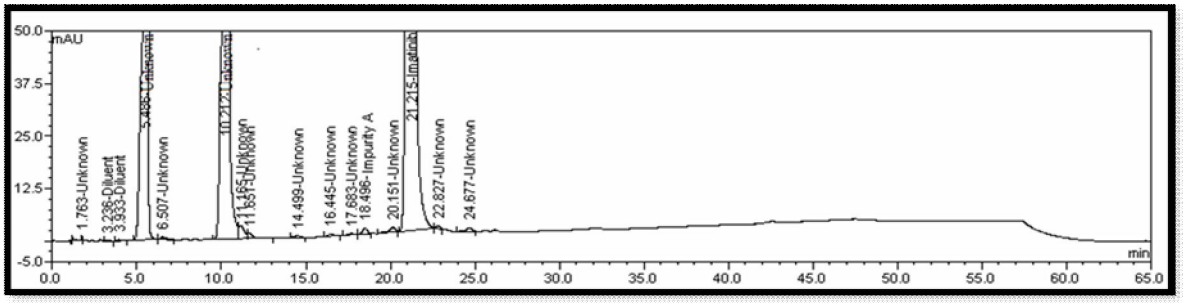
11.201
14.231
20.986-Imatinib
31.730
min

(G)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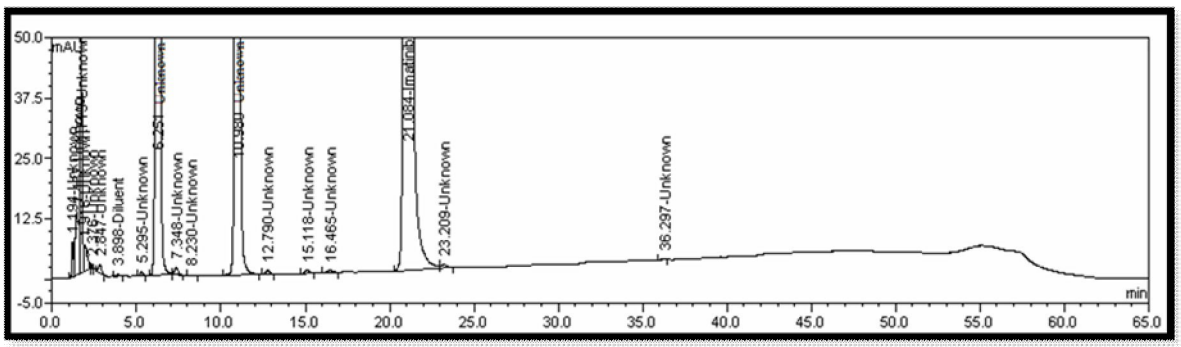
22.827-Unknown
24.677-Unknown
min

(H)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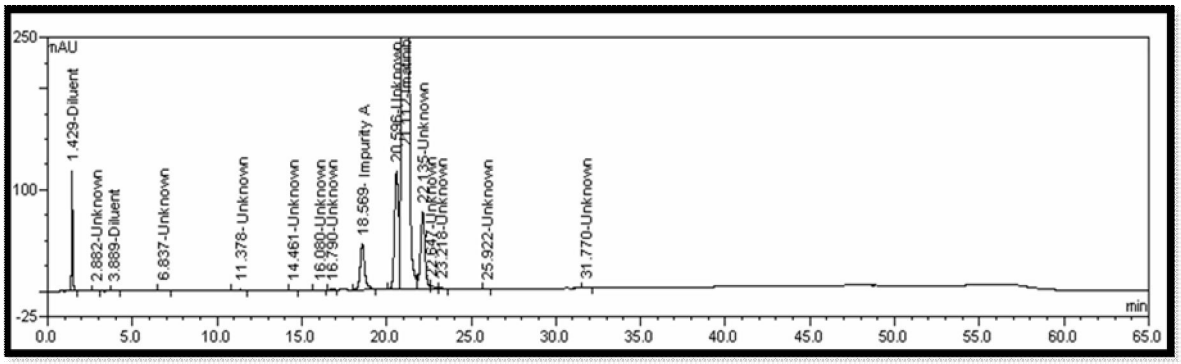
min

(I)



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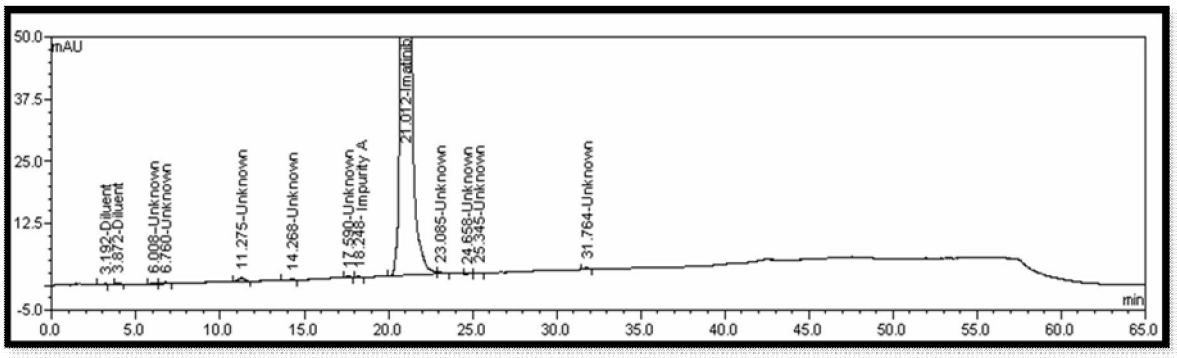

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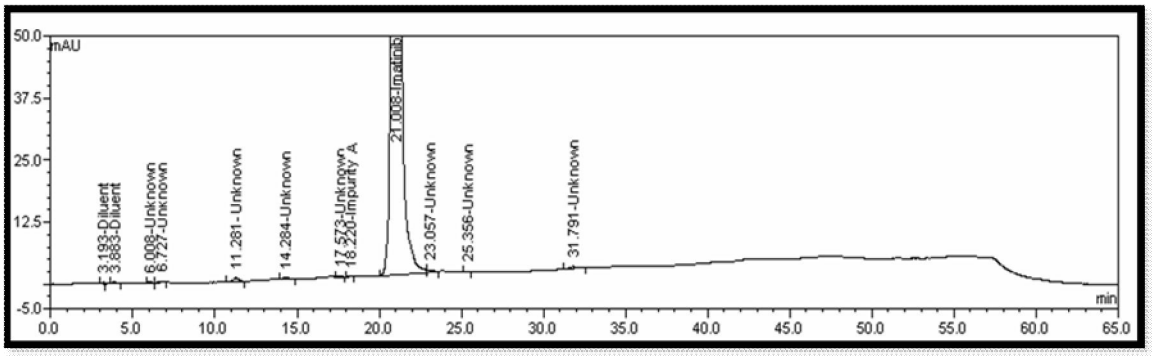

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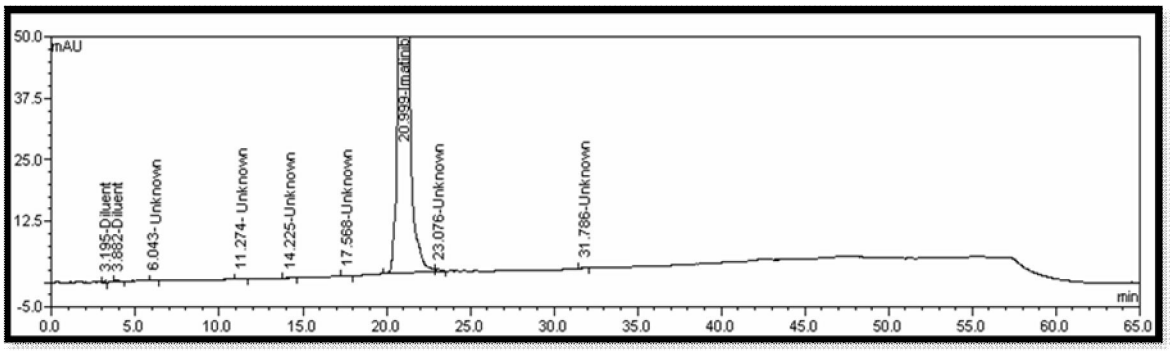


Figure 3: Chromatograms for specificity and force degradation studies (A) Diluent (B) Placebo preparation (C) diluted standard preparation (D) Impurity-A preparation for identification (E) Sample preparation spiked with known impurity-A (F) Sample preparation (Test as such) (G) Acid hydrolysis (H) Alkali hydrolysis (I) Peroxide oxidation degradation (J) Thermal degradation (K) Photolytic degradation (L) water hydrolysis

Imatinib was dropped to 85.4% in acid hydrolysis and degradation peaks were observed in chromatogram and a major degradation products were observed an unknown impurities with area of 5.911% and 8.585% at RRT (relative retention time) about 0.25 and 0.48 respectively. Imatinib was dropped to 83.1% in alkali hydrolysis and degradation peaks were observed in chromatogram and a major degradation products were observed an unknown impurities with area of 7.096% and 9.484% at RRT about 0.29 and 0.52 respectively. Imatinib was dropped to 88.1% in peroxide oxidation degradation and a major degradation product was observed an unknown impurities with area of 3.769% and 5.120% at RRT about 1.05 and 0.98 respectively. Imatinib was dropped to 99.6% in thermal degradation and a major degradation product was observed an unknown impurity with area of 0.044% at RRT about 0.53. Imatinib was dropped to 98.0% in water hydrolysis and a major degradation product was observed an unknown impurity with area of 0.029% at RRT about 1.10. In photolytic degradation, Imatinib a major degradation product was observed an unknown with area of 0.050% at RRT about 0.53. Spectra of Imatinib peak, known impurity-A and unknown impurities degradation products were investigated for spectral purity in the chromatogram for all degraded samples and standards and found spectrally pure. Chromatograms of force degradation studies were presented in Figure 3. The results of force degradation studies data are given in Table 3.

Table 3: Force degradation study data

Degradation conditions	%Impurity-A	%Single maximum unknown impurity	%Total Impurities	%Assay	Mass balance
Test as such	ND	0.010 (RRT 1.51)	0.041	100.0	100.0
Acid hydrolysis	0.074	8.585 (RRT 0.48)	15.153	84.4	99.6
Alkali hydrolysis	ND	9.484 (RRT 0.52)	18.695	80.5	99.2
Peroxide oxidation degradation	2.238	5.120 (RRT 0.98)	13.696	85.6	99.3
Thermal degradation	0.006	0.044 (RRT 0.53)	0.146	99.6	99.7
Photolytic degradation	0.008	0.050 (RRT 0.53)	0.177	99.0	99.2
Water hydrolysis	ND	0.029 (RRT 1.10)	0.086	98.0	98.1

ND – Not detected, RRT - Relative retention time.

3.2.2. Stability of analyte in solution

In diluted standard preparation, percentage relative standard deviation of Imatinib peak area is well within the limit up to 48 hours from the initial area.In sample preparation spiked with known impurity-A, absolute difference in individual impurity and total impurities is well within the limit up to 48 hours from the initial results.Hence, the stability of analyte in solution is established for diluted standard preparation and sample preparation spiked with known impurity-A stable up to 48 hours at 23-27°C and not protected from light.The results of stability of analyte in solution data are given in Table 4.

Table 4: Stability of analyte in solution study data

Time Interval	Diluted standard preparation	Sample preparation spiked with known impurity-A					
		Impurity-A		Single unspecified impurity		Total impurities	
	%RSD	%Impurity	Absolute difference	%Impurity	Absolute difference	%Impurity	Absolute difference
Initial	NA	0.230	N.A.	0.016	N.A.	0.246	N.A.
8 hours	1.25	0.227	0.00	0.013	0.00	0.240	0.01
16 hours	1.29	0.228	0.00	0.012	0.00	0.240	0.01
24 hours	1.17	0.227	0.00	0.012	0.00	0.239	0.01
36 hours	1.15	0.231	0.00	0.017	0.00	0.248	0.00
48 hours	1.19	0.233	0.00	0.016	0.00	0.249	0.00

3.2.3. Limit of detection (LOD) & Limit of quantification (LOQ)

For LOD and LOQ determination, LOD and LOQ value of Imatinib and known impurity-A are presented in Table 5.Linearity curve ofknown impurity-A and Imatinib are presented in Figure 4 and Figure 5 respectively.

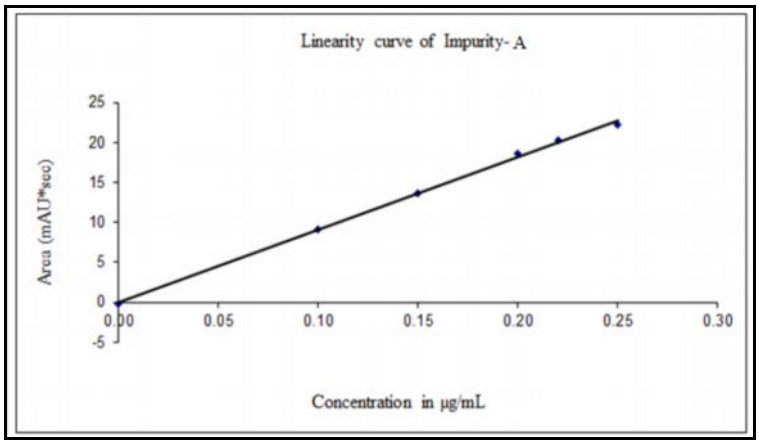


Figure 4: Linearity curve of Impurity-A for LOD and LOQ determination

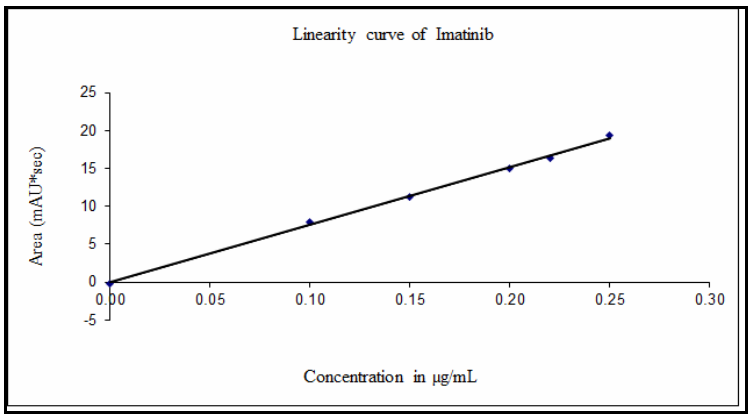


Figure 5: Linearity curve of Imatinib for LOD and LOQ determination

Table 5: LOD and LOQ determination

	Linearity table for Impurity-A		Linearity table for Imatinib	
Linearity level in %	**Conc. in µg/ml**	**Area**	**Conc. in µg/ml**	**Area**
10	0.100	9.0782	0.100	7.9626
15	0.150	13.6622	0.150	11.2452
20	0.200	18.6311	0.200	15.0301
22	0.220	20.2263	0.220	16.4046
25	0.250	22.2191	0.250	19.3334
Slope (S):		89.63324	74.77737	
Residual standard deviation (σ):		0.40109	0.35271	
LOD values in µg/ml:		0.015	0.016	
LOD values in % against test concentration:		0.003	0.003	
LOQ values in µg/ml:		0.045	0.047	
LOQ values in % against test concentration:		0.009	0.009	

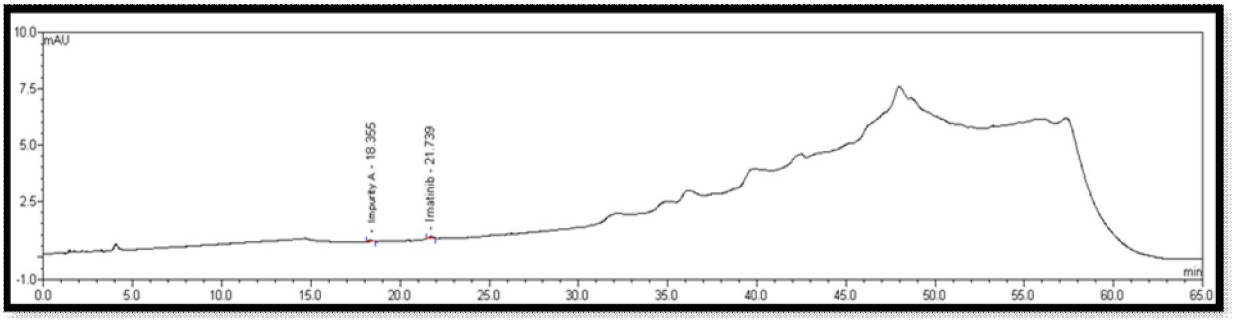
Table 6: LOD and LOQ confirmation

	LOD solution		LOQ solution	
No. of injection	**Impurity-A (Conc.: 0.015 µg/ml)**	**Imatinib (Conc.: 0.016 µg/ml)**	**Impurity-A (Conc.: 0.045 µg/ml)**	**Imatinib (Conc.: 0.047 µg/ml)**
	S/N ratio	**S/N ratio**	**S/N ratio**	**S/N ratio**
1	5.9	7.4	20.5	19.6
2	6.8	8.8	18.9	19.9
3	6.9	7.8	20.1	21.0
4	6.2	8.9	25.8	25.8
5	7.1	10.5	17.7	19.4
6	7.0	8.8	20.0	20.6
%RSD	NA	NA	3.54	3.02

NA=Not applicable

For LOD and LOQ confirmation, the concentration in µg/ml with signal to noise ratio of at least 3 was considered as LOD and concentration in µg/ml with signal to noise ratio of at least 10 was considered as LOQ. The LOD and LOQ confirmation results of Imatinib and known impurity-A are presented in Table 6. Chromatograms for LOD and LOQ solution are given in Figure 6.

(A)



(B)

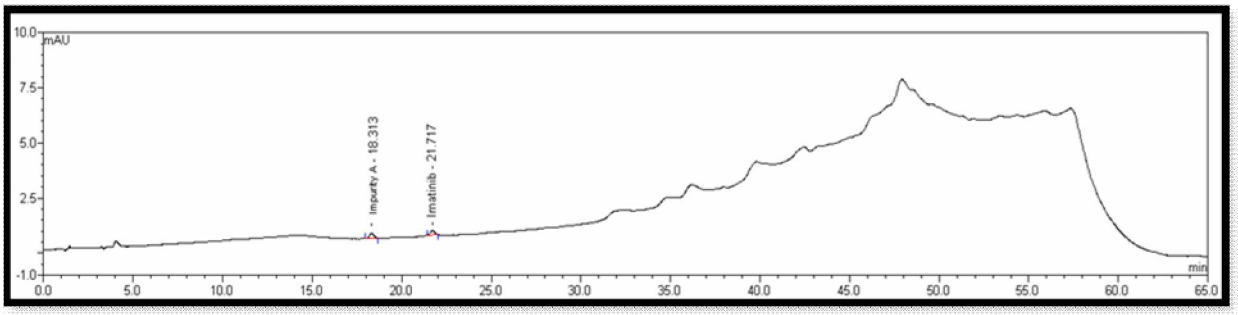


Figure 6: Chromatograms for (A) LOD solution (B) LOQ solution

3.2.4. Linearity

Linearity curve of known impurity-A and Imatinib are presented in Figure 7 and Figure 8 respectively. The results of linearity for known impurity-A and Imatinib are given in Table 7 and Table 8 respectively.

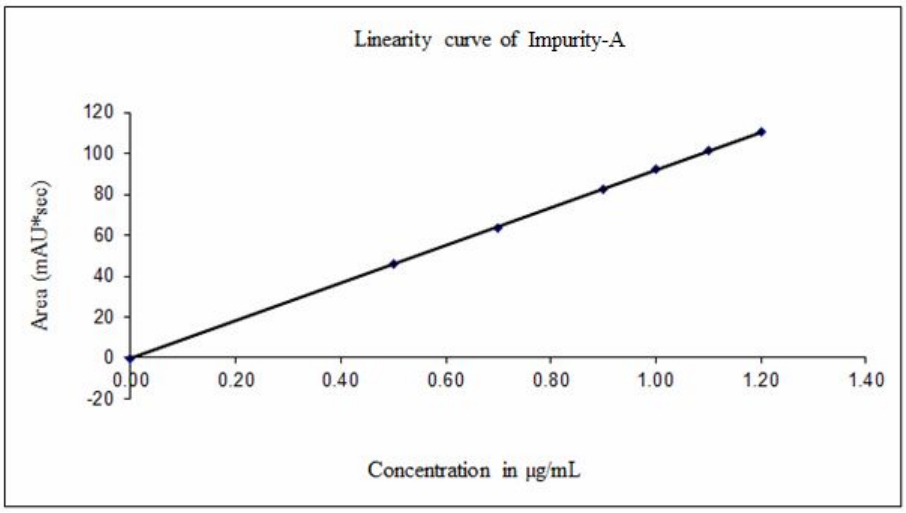


Figure 7: Linearity curve of Impurity-A

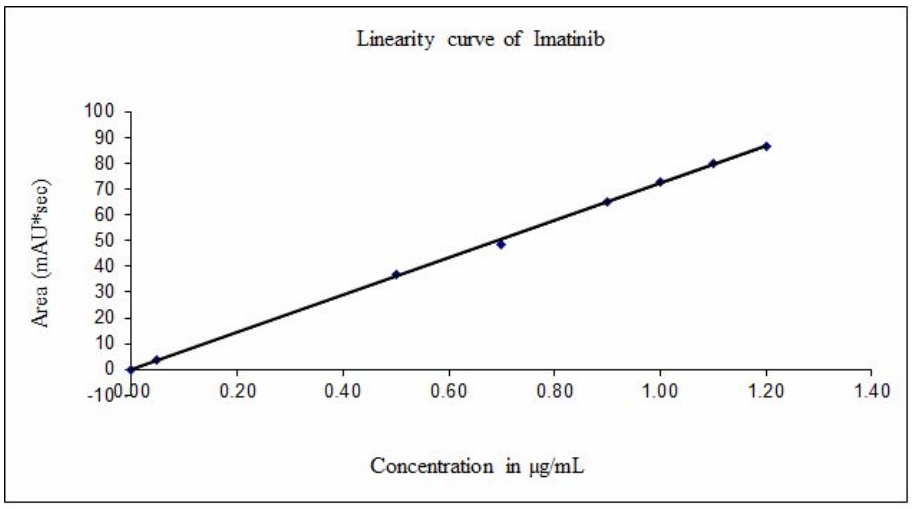


Figure 8: Linearity curve of Imatinib

Table 7: Linearity table for Impurity-A

Linearity levelin %	Concentration in µg/mL	Area	Response Factor
LOQ	0.045	4.1949	93.220
50	0.500	46.1635	92.327
70	0.700	63.6937	90.991
90	0.900	82.4006	91.556
100	1.000	92.1603	92.160
110	1.100	101.7297	92.482
120	1.200	110.5328	92.111
		Correlation Coefficient:	0.99994
		R^2 value:	0.99988
		Y-Intercept:	-0.15636
		Slope:	92.18917
		%RSD of Response factor:	0.61
		% Y-Intercept bias at 100 % level:	-0.2
		Relative response factor:	0.78

Table 8: Linearity table for Imatinib

Linearity levelin %	Concentration in µg/mL	Area	Response Factor
LOQ	0.047	3.5705	75.968
50	0.500	36.7201	73.440
70	0.700	48.6975	69.568
90	0.900	65.0804	72.312
100	1.000	72.7041	72.704
110	1.100	79.7818	72.529
120	1.200	86.4375	72.031
		Correlation Coefficient:	0.99958
		R^2 value:	0.99916
		Y-Intercept:	-0.03788
		Slope:	72.19700
		%RSD of Response factor:	1.84
		% Y-Intercept bias at 100 % level:	-0.1
		Relative response factor:	1.00

3.2.5. Precision

3.2.5.1. System precision

System suitability parameters are well within the limit. System suitability acceptance Criteria and results are given in Table 9.

Table 9: System precision

System suitability parameters	Results	Acceptance Criteria
%Relative standard deviation of replicate injections for Imatinib peak area	0.45%	Not more than 5.00%
Theoretical plates (by tangent method) for Imatinib peak	25533	Not less than 2000
Tailing factor for Imatinib peak	1.21	Not more than 2.0
% Relative standard deviation of replicate injections for retention time for Imatinib peak	0.20	Not more than 1.0

3.2.5.2. Method precision and Intermediate precision

Percentage relative standard deviation of six results for each individual impurity and total impurities is well within the limit. Absolute difference in individual impurity and total impurities is well within the limit from the impurity values obtained from method precision study. Method precision and intermediate precision results are given in Table 10.

Table 10: Comparison between method precision and intermediate precision

Set No.	%Impurity results					
	Impurity-A		Single unspecified impurity		Total impurities	
	Method precision	Intermediate precision	Method precision	Intermediate precision	Method precision	Intermediate precision
1	0.242	0.239	0.011	0.014	0.253	0.253
2	0.240	0.236	0.012	0.012	0.252	0.248
3	0.239	0.238	0.012	0.011	0.251	0.249
4	0.240	0.237	0.011	0.012	0.251	0.249
5	0.243	0.236	0.012	0.013	0.255	0.249
6	0.242	0.237	0.012	0.014	0.254	0.251
Mean	0.241	0.237	0.012	0.013	0.253	0.250
%RSD	0.64	0.49	4.43	9.56	0.65	0.73
Absolute difference	0.00		0.00		0.00	

3.2.6. Accuracy (Recovery)

Recovery of known impurity-A and Imatinib is well within the limit. Percentage relative standard deviation for recovery 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 11.

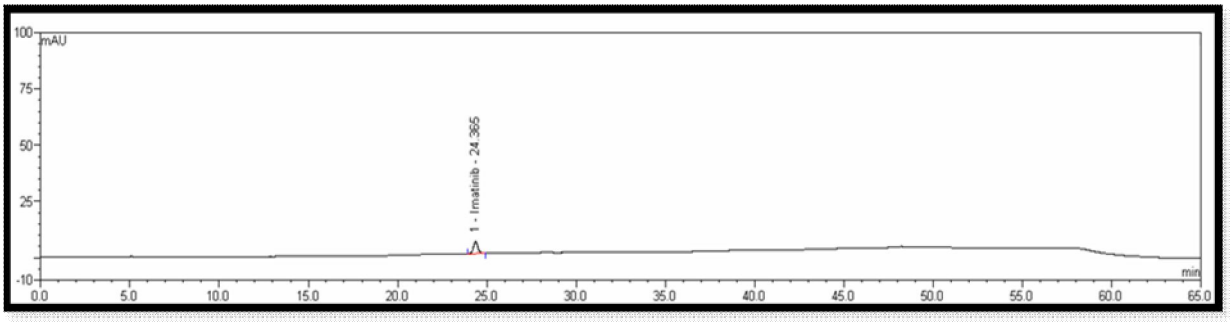
Table 11: Accuracy (Recovery)

		Recovery data forImpurity-A			Recovery data forImatinib		
Set No.	Recovery level	Theoretical amount in µg/mL	Recovered amount in µg/mL	%Recovery	Theoretical amount in µg/mL	Recovered amount in µg/mL	%Recovery
Set-1	LOQ	0.04508	0.04685	103.9	0.04712	0.04813	102.1
Set-2			0.04686	103.9		0.04715	100.1
Set-3			0.04692	104.1		0.04602	97.7
			Mean:	104.0		Mean:	100.0
			%RSD:	0.10		%RSD:	2.24
Set-1	50%	0.50075	0.50509	100.9	0.50053	0.51005	101.9
Set-2			0.50734	101.3		0.50723	101.3
Set-3			0.51053	102.0		0.50315	100.5
			Mean:	101.4		Mean:	101.3
			%RSD:	0.54		%RSD:	0.68
Set-1	100%	1.00064	1.00961	100.9	0.10005	0.10266	102.6
Set-2			1.00782	100.7		0.10197	101.9
Set-3			1.00758	100.7		0.10379	103.7
			Mean:	100.8		Mean:	102.8
			%RSD:	0.11		%RSD:	0.89
Set-1	120%	1.20097	1.20565	100.4	1.19906	1.19561	99.7
Set-2			1.20893	100.7		1.19451	99.6
Set-3			1.20844	100.6		1.19297	99.5
			Mean:	100.6		Mean:	99.6
			%RSD:	0.15		%RSD:	0.11
			Over all Mean:	101.7		Over all Mean:	100.9
			Over all %RSD:	1.54		Over all %RSD:	1.42

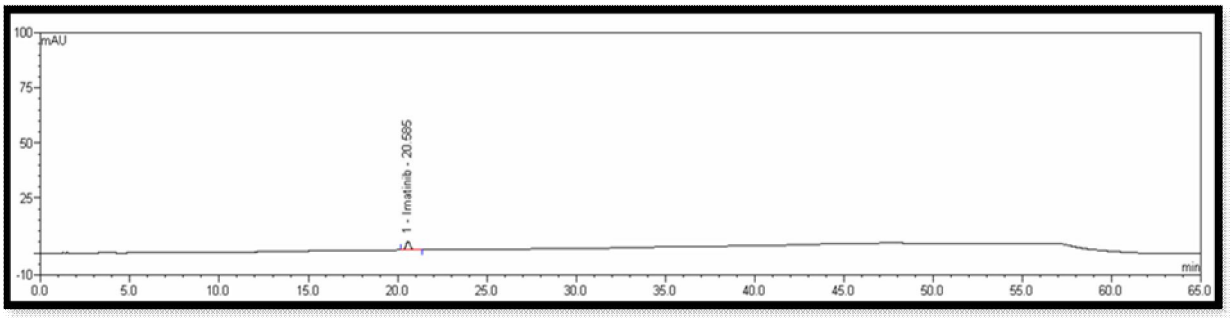
3.2.7. Robustness

No significant effect was observed on system suitability parameters such as percentage relative standard deviation of peak area, tailing factor and theoretical plates when small but deliberate changes were made to chromatographic conditions. The data of robustness are given in Table 12 and Table 13. Specimen chromatograms for robustness parameters are given in Figure 9.

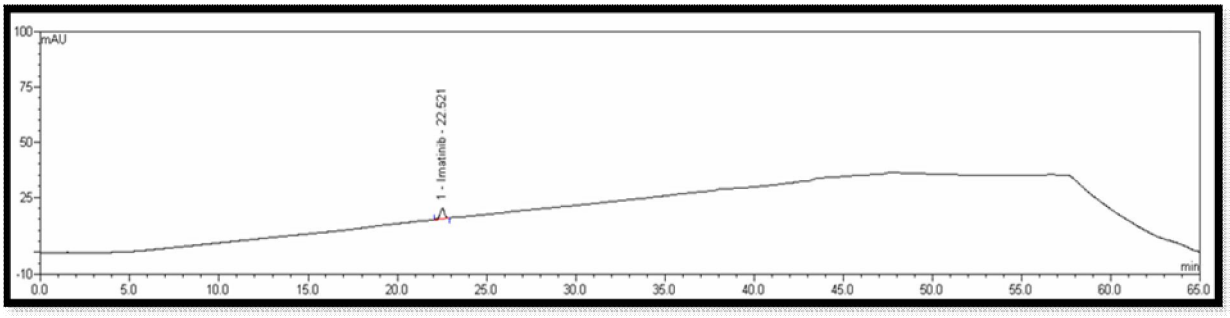
(A)



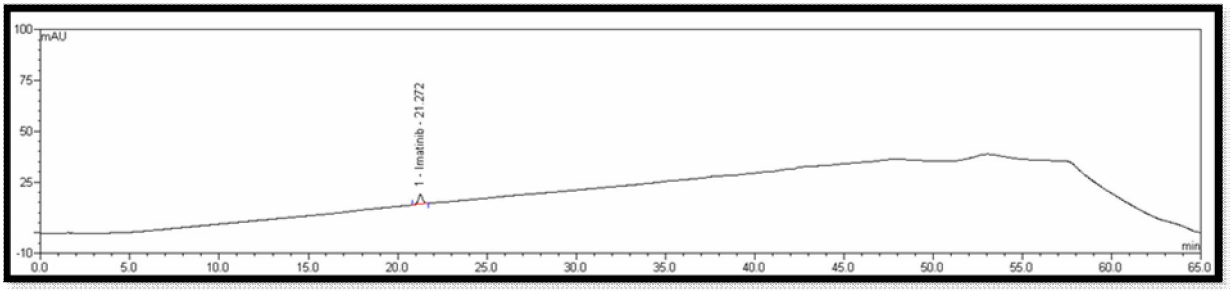
(B)



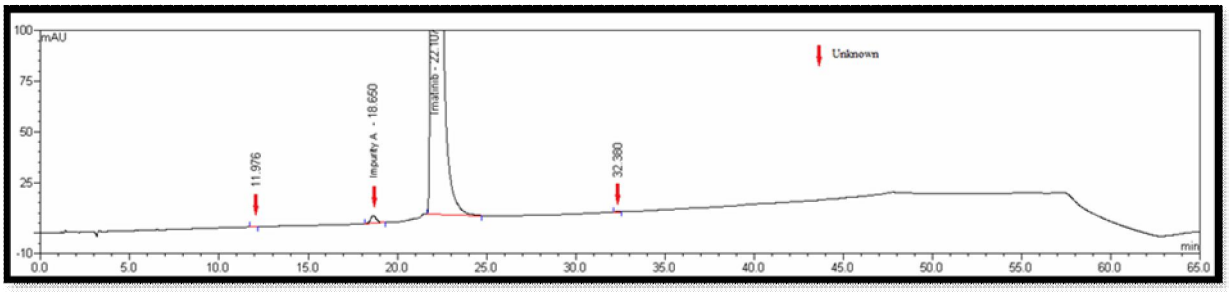
(C)



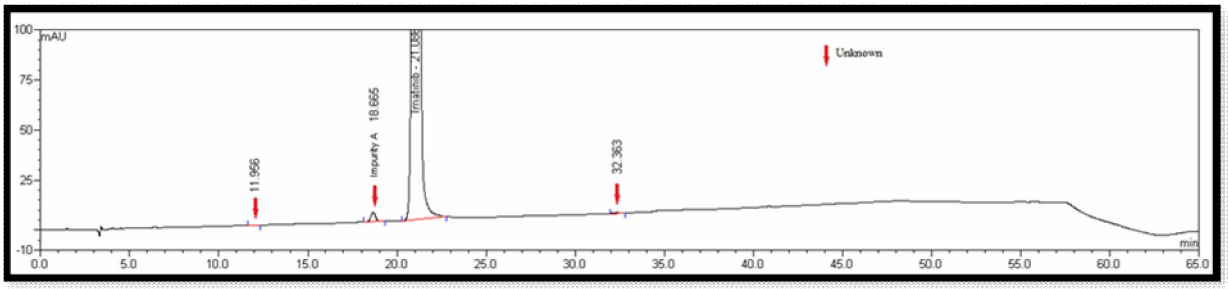
(D)



(E)


mAU
Unknown
11.976
Impurity A - 18.650
Imatinib - 22.107
32.380
min

(F)


mAU
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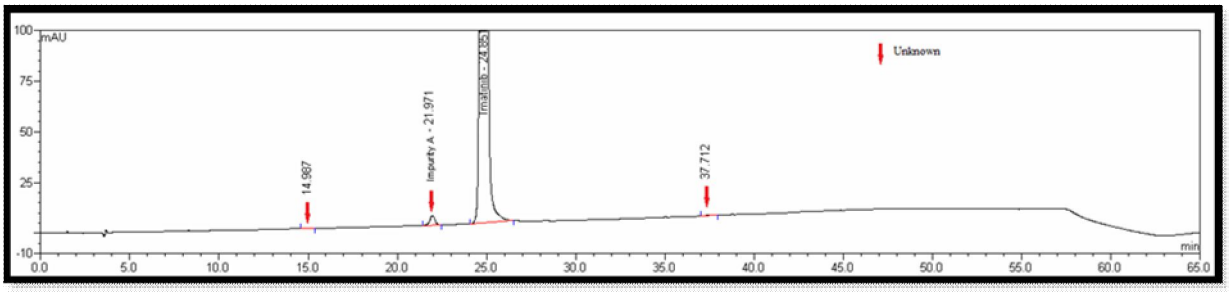
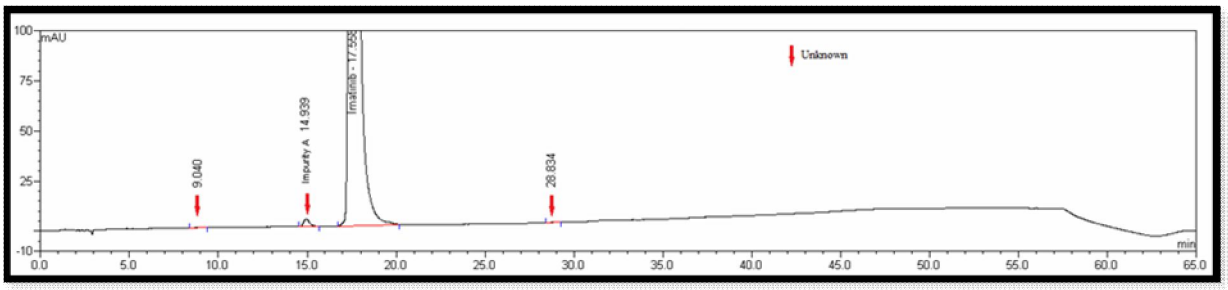
Impurity A 18.665
Imatinib - 21.188
32.363
min

(G)


mAU
Unknown
14.967
Impurity A - 21.971
Imatinib - 24.895
37.712
min

(H)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Impurity A 14.939
Imatinib - 17.558
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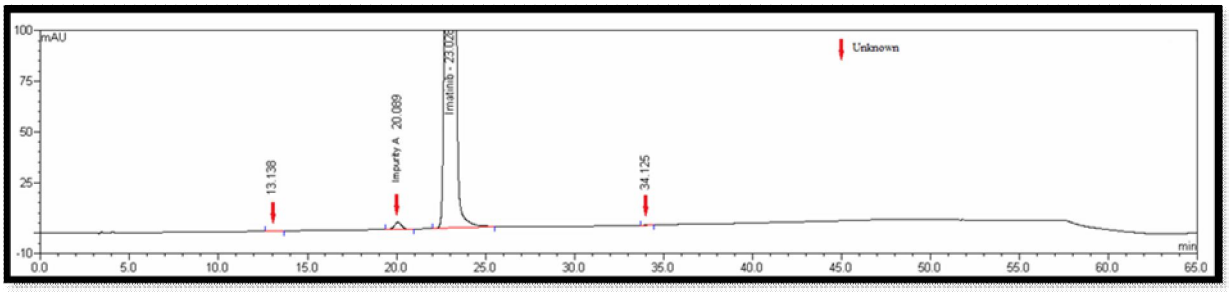
min

(I)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Impurity A 20.089
Imatinib - 23.028
34.125
min

(J)

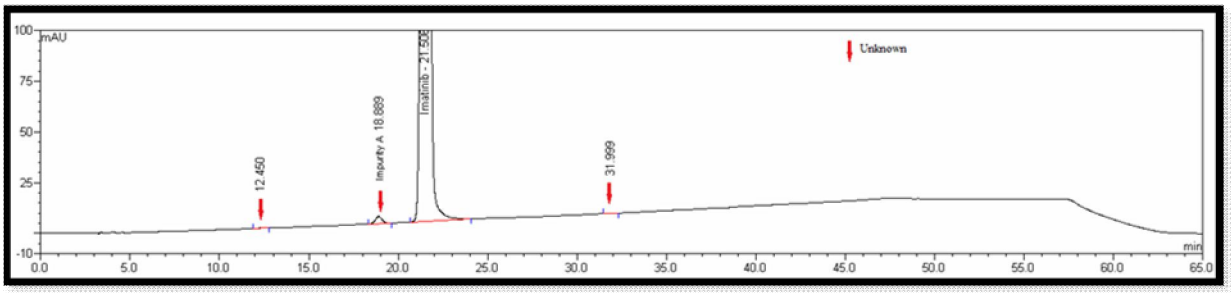

Figure 9: Specimen chromatograms for robustness parameters
(A) Variation in flow rate (- 0.2 mL/minute): 0.8 mL/minute (Actual 1.0 mL/minute)
(B) Variation in flow rate (+ 0.2 mL/minute): 1.2 mL/minute (Actual 1.0 mL/minute)
(C) Variation in column oven temperature (- 2°C): 25°C (Actual 27°C)
(D) Variation in column oven temperature (+ 2°C): 29°C (Actual 27°C)
(E) Variation in pH of mobile phase buffer: (- 0.2 pH): 2.8 pH (Actual 3.0 pH)
(F) Variation in pH of mobile phase buffer: (+ 0.2 pH): 3.2 pH (Actual 3.0 pH)
(G) Variation in mobile phase-A composition (-5% methanol solvent)
(H) Variation in mobile phase-A composition (+5% methanol solvent)
(I) Variation in mobile phase-B composition (-2% methanol solvent)
(J) Variation in mobile phase-B composition (+2% methanol solvent)

Table 12: Comparison of system suitability between precision and altered robustness parameters

Robustness Conditions	%RSD of Retention time	%RSD of peak area	Theoretical plates	Tailing factor
System precision (Precision study)	0.20	0.45	25533	1.21
Flow rate: 0.8 ml/minute	0.10	0.92	50821	1.03
Flow rate: 1.2 ml/minute	0.07	1.01	39564	1.21
Column oven temp.: 25°C	0.05	0.81	45905	1.13
Column oven temp.: 29°C	0.05	0.84	41101	1.16
Mobile phase buffer pH: 2.8	0.09	1.25	29216	1.21
Mobile phase buffer pH: 3.2	0.15	1.05	39484	1.01
Mobile phase-A composition: +5% Methanol	0.10	1.55	17580	1.21
Mobile phase-A composition: -5% Methanol	0.15	0.79	65852	1.05
Mobile phase-B composition: +2% Methanol	0.09	1.61	20559	1.25
Mobile phase-B composition: -2% Methanol	0.08	0.92	22528	1.14

Table 13: Comparison of results for method precision and altered robustness parameters

Robustness parameters	Impurity-A		Any other single unknown impurity		Total impurities	
	% Impurity	Absolute difference	% Impurity	Absolute difference	% Impurity	Absolute difference
Method precision	0.241	N.A	0.012	N.A	0.253	N.A
Mobile phase buffer pH: 2.8	0.219	0.02	0.011	0.00	0.226	0.03
Mobile phase buffer pH: 3.2	0.236	0.01	0.017	0.01	0.260	0.01
Mobile phase-A composition: +5% Methanol	0.232	0.01	0.015	0.00	0.241	0.01
Mobile phase-A composition: -5% Methanol	0.235	0.01	0.018	0.01	0.247	0.01
Mobile phase-B composition: +2% Methanol	0.240	0.00	0.012	0.00	0.254	0.00
Mobile phase-B composition: -2% Methanol	0.229	0.01	0.011	0.00	0.235	0.02
Extraction time: 10 minute	0.232	0.01	0.017	0.01	0.248	0.01
Extraction time: 20 minute	0.233	0.01	0.012	0.00	0.244	0.01

4. Conclusion

A stability indicating reverse phase high performance liquid chromatography method was effectively developed and validated for the related substances determination of process impurities and degradation products of Imatinib in tablet dosage form. The developed method was validated in accordance with ICH guidelines and recommended stress condition. The method validation results has been proved that the method to be specific, precise, linear, accurate and robust with stability indicating power. Hence, the method can be used for routine quality control analysis and also stability sample analysis. The degradation study results shows that the drug is stable at thermal, photolytic and water hydrolysis conditions.

Acknowledgement

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