International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
https://www.theaspd.com/ijes.php

A Novel RP-HPLC Method For Imatinib Mesylate Impurity Profiling

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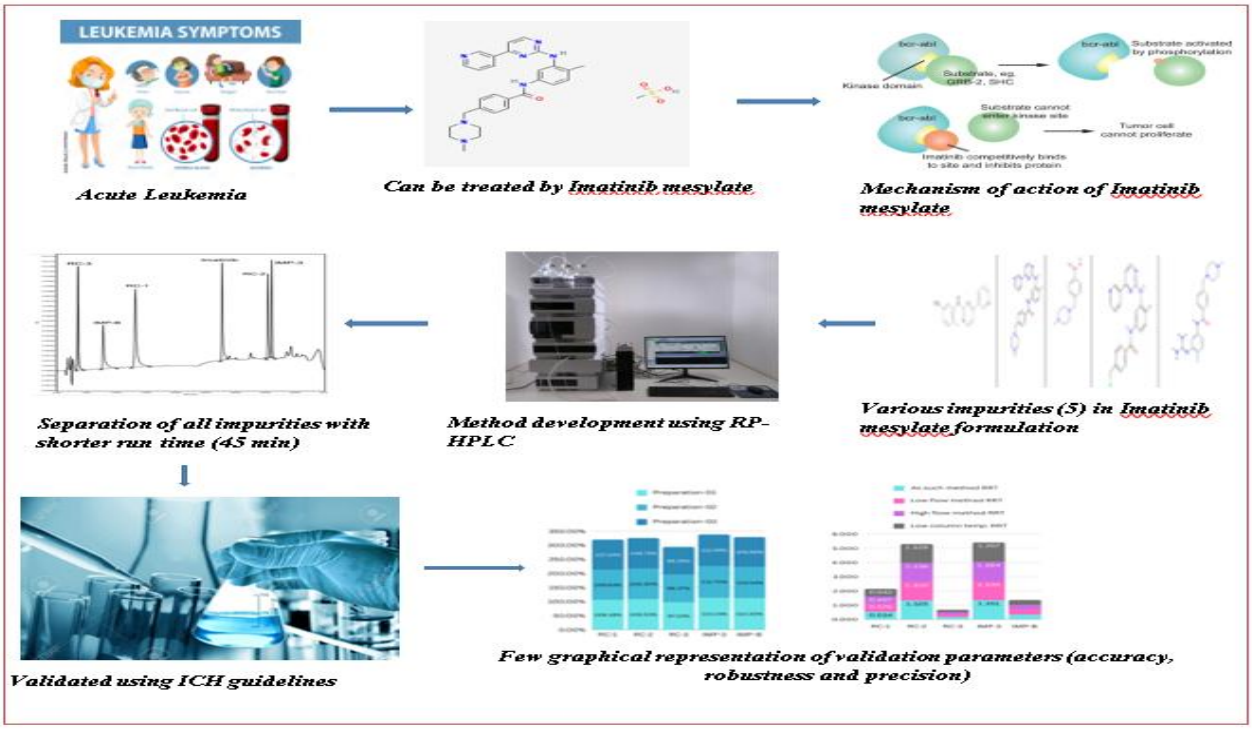


Submission date: 29-05-2025; Acceptance date: 10-07-2025

Abstract
Imatinib methane sulfonate is a mesylate salt, specifically the monomesylate salt of imatinib. Employed in the management of chronic myelogenous leukaemia, various malignancies, haematological disorders, and gastrointestinal stromal tumours. It functions as an antineoplastic agent, an inducer of apoptosis, a tyrosine kinase inhibitor, and an agent against coronaviruses. The primary aim of the research was to elucidate the process of impurity separation, accomplished through the development and validation of an analytical method for

quantifying the impurities present in Imatinib Mesylate. This method presents a distinct advantage, as there are only a limited number of analytical techniques documented for the quantification of Imatinib and its related substances in both bulk and pharmaceutical formulations. Five impurities were successfully isolated, and a method was developed and validated utilizing HPLC on a Zodiac C18 (150 mm × 4.6 mm, 5 µm) column within a gradient elution scheme. The wavelength for detection was established at 240 nm. The retention time for the drug was recorded at 27.2 minutes, with the analysis concluding within a span of 45 minutes. The samples are subjected to the forced degradation conditions—hydrolysis, oxidation, acidic, basic, and dry heat specified by the International Conference on Harmonization. The technique distinguished between unknown substances and five known impurities. Related Compound 1, Related Compound 2, Related Compound 3, Impurity 3, and Impurity B. According to the International Conference on Harmonization's guidelines, the method was validated in terms of specificity, linearity, precision, accuracy, and limits of detection and quantification.

Keywords Imatinib Mesylate, Validation, Related compound-1, Related compound-2, Related compound-3, Impurity-3, Impurity-B, Forced degradation.

1. Introduction

Imatinib mesylate functions as a protein-tyrosine kinase inhibitor, specifically targeting the BCR-ABL tyrosine kinase, which is the persistently active tyrosine kinase resulting from the Philadelphia chromosome anomaly in chronic myeloid leukaemia (CML). (national centre for biotechnology information, n.d.) (1). Imatinib exerts its inhibitory effects on the BCR-ABL protein. Protein tyrosine kinases collectively phosphorylate specific amino acids on substrate proteins, initiating signal transduction that alters cellular biology, including growth, differentiation, and apoptosis; persistent activation due to mutations or other factors can result in malignancy. Inhibiting this essential activity has been demonstrated to trigger subsequent apoptosis without additional differentiation. Imatinib, as a therapeutic agent, inhibits the constitutive activity of protein tyrosine kinase by functioning as a competitive inhibitor of the ATP binding site of ABL, thereby triggering apoptosis in leukemic cells (BJ, 2001) (2). Imatinib is available in 100 mg and 400 mg tablet forms and has been authorized for oral use by the FDA

Imatinib is a benzamide synthesized through the formal condensation of the carboxy group of 4-[4-methylpiperazin-1-yl) methyl]benzoic acid with the primary aromatic amino group of 4-methyl-N(3)-[4-(pyridine-3-yl)pyrimidin-2-yl]benzene-1,3-diamine. Imatinib is primarily a neutral compound that exhibits lipophilicity at a pH of 7.4. It possesses a molecular weight of 493.603 g/mol and pKa values of 8.07, 3.73, 2.56, and 1.52. It is insoluble in n-octanol, acetone, and acetonitrile; freely soluble to extremely soluble in dimethyl sulfoxide, methanol, and ethanol; soluble in aqueous buffers with a pH less than 5.5, however slightly soluble in neutral to alkaline aqueous buffers. This is a Class-1 compound characterized by elevated permeability and solubility. (pubchem, n.d.) (3, 5) Figure 1 illustrates the structural composition of Imatinib along with its associated impurities.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
https://www.theaspd.com/ijes.php

Figure: 1 Structure of Imatinib and its associated Impurities

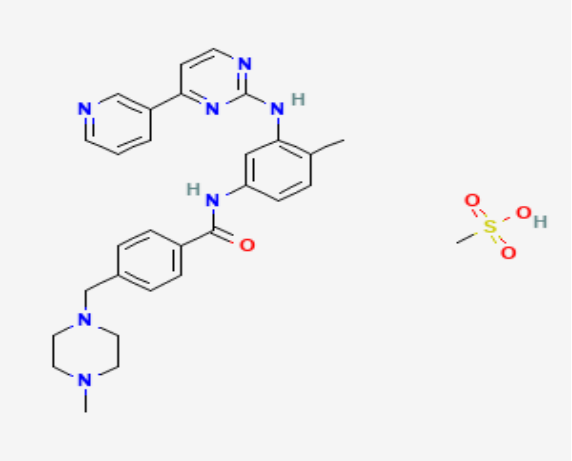
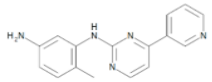
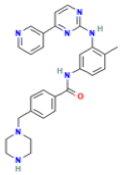
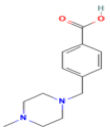
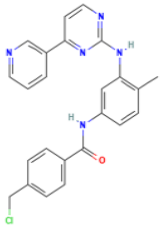
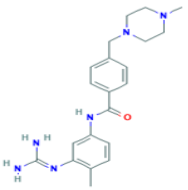


Table 1: Imatinib Mesylate (pubchem)(4,5)

RC-1	RC-2	RC-3	IMP-3	IMP-B
N-(2-methyl-5-aminophenyl)-4-(93-pyridyl)-2-pyrimidine	N-(4-methyl-3-[[4-(3-pyridinyl]-amino]phenyl]4-methyl benzamide methane sulfonate	4-(4-methyl piperazinyl methyl)benzoic acid dihydrochloride	N-[4-Methyl-3-(4-pyridin-3-yl-pyrimidin-2ylamino)-phenyl]-4-chloromethlyBenzamide	N-[3-[(Aminoiminomethyl)amino]-4-methylphenyl]-4-[(4-methyl-1-piperazinyl)methyl]benzamide
				
Process Related Impurity	Degradant	Degradant	Process Related Impurity	Process Related Impurity

A search of the literature disclosed that number of analytical methods reported for determination of Imatinib in bulk and finished product by simple UV spectrophotometric (patil s, 2013) (6), HPLC (p.sandhya, 2013)(7), (Vrushali Kharate) (8) and HPTLC (mahesh s. wajurkar, 2015) (9) techniques. Only few analytical methods reported for the quantification of Imatinib and its related substances in bulk and pharmaceutical formulations. Singh A. et al presented a validated LC method with two impurities with run time of 80 minutes. (Singh A, 2022) (10). Sensitive and selective liquid mass a spectrophotometric liquid-tandem chromatography (LC-MS/MS) method was developed and validated for one Genotoxic Impurity A. (Vaibhav Bhatt, 2013) (11) Bielejewska A et al. reported degradation of Imatinib in hydrolytic, oxidative, photolytic conditions and characterization of oxidative impurities. (A. bielejewsha, 2007) (12) Imatinib not published in any official monographs and there is necessity to develop the stability indicating method with intend to work in quality control. Some of the literature provide information about the impurity's studies in HPLC (Andal, 2024) (13), quantification of drug in rat serum in HPLC (Bende, 2010) (14), estimation of drug with its dimer impurity by HPLC (Kuna, 2018) (15), Stability studies by UV- Spectrophotometer method (Swetha, 2022) (16), determination of Imatinib by ion pair complex colorimetry (Rajan, 2024) (17), stability indicating method development and validation of drug by RP-HPLC (Shah, 2015) (18), a validated LC method for the drug (Vivekanand, 2003) (19), residual solvent determination by gas chromatography in API (Sojitra, 2019) (20), Table 2 illustrate the research gaps, issues and problems for future work from referred papers.

Table 2: Research gaps, issues and problems for future work from papers referred.

Journal Name	Title of the Paper	Problems from referred paper
Indian Journal of Pharmaceutical Sciences *Indian J Pharm sci 2022;84(2):465-476*	Development and Validation of Stability Indicating High Performance Liquid Chromatography Method for Related Substances of Imatinib Mesylate	Only 2 impurities considered with 60 min run time
International Journal of Pharma Sciences and Research (IJPSR) *G. Sathwik et al.Vol.6 No.4 Apr 2015*	Analytical method development and validation of solid dosage form of antineoplastic drug Imatinib Mesilate by RP-HPLC.	Assay by HPLC method with 5 min run time published
WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES *P.Sandhya et al,vol 3 Issue1,682-688*	Method development and validation of Imatinib Mesylate in pharmaceutical dosage form by RP-HPLC	Assay by HPLC method with 5 min run time published
Journal of Chromatography *K.L,Tan et al/J.Chromatogr.B 879(2011) 3583-3591*	Method development and validation for the simultaneous determination of Imatinib Mesylate and N-desmethyl Imatinib using rapid resolution high performance liquid chromatography coupled with UV-detection.	UPLC Method with one impurity only published.

The RP-HPLC method exhibits remarkable specificity, facilitating the precise identification and quantification of Imatinib Mesylate along with its impurities, even at minimal concentrations. This specificity is crucial for ensuring the quality and safety of pharmaceutical products. Gradient elution can separate a sample containing compounds with a wide range of polarities in less time without losing resolution in the earlier peaks or excessive broadening of later peaks. However, maintaining a constant flow rate with continuous changes in mobile phase composition is more difficult. Gradient elution is used in both preparative and large-scale chromatography to separate compounds (Ryan, 1998) (21).

Impurity profiling is crucial for adhering to the stringent regulatory standards established by agencies like the FDA and EMA. These regulations require the precise identification and quantification of impurities to guarantee the safety and efficacy of pharmaceutical products. Specific impurities may expedite degradation or disrupt formulations, thereby influencing the product's effectiveness. Comprehending these dynamics facilitates the identification of suitable storage conditions essential for preserving quality (24)- (25) Forced degradation plays a crucial role in elucidating degradation pathways, thereby informing formulation strategies and enhancing predictions regarding shelf-life.

The objective of this work was to develop a simple and economical HPLC method for quantification of Imatinib with its five related substances in bulk and pharmaceutical formulations. (Tan, 2011) (22), (Dass, 1997) (23), Developed a gradient reversed phase chromatographic method with a run time of 45 min and performed analytical method validation as per ICH recommendations (26)- (27). The developed method is confirmed as a stability-indicating method (SIM) and is suitable for use in the stability monitoring of the drug product Imatinib mesylate (28)- (30).

2. Experimental

2.1. Materials

Imatinib Mesylate, along with five impurities, with characterization parameters proton NMR, FT-IR and molecular weight by Mass (MS) is obtained from Laurel Pharma Labs located in Hyderabad, India. Imatinib Mesylate working standard with potency 99.4% used for the study which is qualified against the USP reference standard. R.C-1 with potency 99.3%, RC-2 with potency 98.9%, RC-3 with potency of 99.1%, IMP-3 of 99.3% and IMP-B of 99.1%. Purified water obtained from the Milli Q water purifying system. The chemicals used in this method were listed in Table 3.

Table 3: List of Chemicals and their Make

Name	Lot Number	Make	Grade
Disodium Hydrogen Orthophosphate	H2065	Rankem	A.R
Ortho phosphoric acid	DHD731446	Merck	A.R
Acetonitrile	M13M732003	Qualigens	HPLC
Water	Not applicable	Milli-Q water	HPLC

2.2. Instrumentation and Chromatographic conditions

Instrument employed is a Waters gradient HPLC system that includes an e2695 solvent delivery system (pump), an auto sampler injector equipped with a 50 µl loop, and a 2998 PDA detector, all functioned with Empower Version 3.2.6 Software. The wavelength of the detector was observed at 240 nm. Solvent A was 0.028 M Disodium hydrogen with pH 2.8 adjusted with orthophosphoric acid. Solvent B was Acetonitrile. Solvent A used as diluent. Instrumental conditions employed in Table 4.

Table 4: Instrumentation conditions

Parameters	Chromatographic conditions
Instrument	Waters gradient HPLC system with e2695 solvent delivery system (pump)
Column	Zodiac C18 150*4.6, 5µm.
Detector	2998 PDA detector

2.3. Preparation of Analytical Solutions:

Preparation of Mobile phase and diluent

Buffer solution is prepared by dissolving 10.07gm of disodium hydrogen phosphate in 1000ml of water to make 0.028M. The solution is adjusted to pH 2.8 with Ortho Phosphoric acid. This solution is used as diluent. For gradient programme 0.028M disodium hydrogen phosphate is taken as solvent A and acetonitrile is taken as solvent B.

Preparation of Standard Solution

A standard stock solution of the drug was prepared by dissolving pure drug in the diluent, to get the solution concentration about 2.5 ppm.

Preparation of sample solution

Triturate the contents of 20 tablets of Imatinib mesylate of 100 mg and from which take weighed 25 mg equivalent sample into a 50 mL volumetric flask, added 20 mL of diluent and sonicated for 30 minutes with intermediate shaking and kept aside for attaining room temperature. After that, make-up up to the mark with diluent and mixed well. Centrifuge a portion of sample at 3000 rpm for 10 minutes and then injected the supernatant solution into the chromatographic system. The final concentration of solution is 500 ppm in diluent.

Placebo Solution

Weighed 25 mg equivalent of placebo into a 50 mL volumetric flask, added 20 mL of diluent and sonicated for 30 minutes with intermediate shaking and kept aside for attaining room temperature. After that, make-up up to the mark with diluent and mixed well. Centrifuge a portion of sample at 3000 rpm for 10 minutes and then injected the supernatant solution into the chromatographic system. The final concentration of solution is 500 ppm in diluent.

2.4. Stress Studies

Both placebo and test samples were utilized in the conducting of forced degradation studies for the purpose of stress analysis. In the process of acid hydrolysis, a 0.1N solution of Hydrochloric acid (HCl) was introduced to both the test solution and the placebo solution. These solutions were maintained at room temperature for a duration of six days, after which the sample preparation procedure was followed accordingly. In the process of base hydrolysis, a 0.1N solution of Sodium Hydroxide (NaOH) was introduced to both the test solution and the placebo solution. These mixtures were maintained in a water bath at a temperature of 60°C for a duration of 24 hours, after which the sample preparation procedure was followed accordingly. In the study of oxidative stress, a 3% solution of hydrogen peroxide (H2O2) was incorporated into both the test and placebo solutions. These solutions were maintained at room temperature for a duration of three hours, after which the sample preparation protocol was followed accordingly. In the study of humidity stress, the completed product sample and the placebo, both in solid form, were subjected to a relative humidity of 75% for approximately 24 hours, after which they were processed according to the established sample preparation protocol. In the investigation of thermal stress, the completed product sample and placebo were maintained at a temperature of 60°C for a duration of 24 hours, after which the sample solution was prepared in accordance with the established sample preparation protocol. In the study of photolytic stress, both the finished product sample and the placebo were subjected to an exposure of 200-Watt hours/m2 for UV light and 1.2 million lux hours for visible light, followed by the

established sample preparation procedure. In addition to the previous stress samples, the control sample, which remains unstressed, will be introduced in sequence for the purpose of measuring the mass balance. In order to establish it as a Stability Indicating Method (SIM), the percentage assay results were calculated from both the stressed samples and the control sample (unstressed sample), alongside the percentage of impurities from the stressed samples to facilitate the computation of mass balance. All stressed samples exhibited a mass balance exceeding 95%, aligning with the established protocol acknowledged as the Stability Indicating Method (SIM).

2.5. Method Validation

The validation of an analytical procedure seeks for proof of its suitability for the quality. To validate an analytical technique, one must demonstrate that it can accurately analyse the drugs and any associated compounds. The HPLC technique that was developed and validated to separate, identify and quantify the sample and the related compounds of Imatinib in the finished product as per the ICH guidelines. The validation of specificity, system precision, method precision, intermediate precision, accuracy (recovery), establishment of LOD and LOQ, precision at LOQ, recovery at LOQ level, linearity, and forced degradation studies was conducted for the determination of impurities and degradation products of Imatinib mesylate using RP-HPLC. Upon completion of the analytical method development, the relative response factor (RRF) was established as a foundation for the concentration method.

2.5.1 System suitability

The chromatographic system must validate the system suitability parameters prior to the initiation of sample analysis. After installing the chromatographic system, stabilize the HPLC equipment for 40 minutes. To evaluate the system suitability, inject one injection of the blank preparation and six replicates of the standard preparation. Subsequently, analyse the chromatograms and evaluate the system's repeatability (% RSD not exceeding 2.0), symmetry factor (not exceeding 2.0), retention factor (not less than 2.0), resolution (not less than 2.0), theoretical plate count (not less than 2000), and signal-to-noise ratio (not less than 100). The method produced satisfactory system suitability results. Table 5 represents the system suitability data.

Table 5: System Suitability Results

Compound	Peak Tailing	%RSD of Imatinib	Theoretical Plate Count	Retention Time (min)
Imatinib	2.4	2.7%	122409	27.181

2.5.2 Precision

In terms of System, Method, and Intermediate precision, precise parameters were demonstrated. System appropriateness criteria are met and system precision is verified by employing a standard solution. Six individual samples were prepared from the homogeneous sample in accordance with the test protocol, either by spiking each impurity separately or by blending the impurity stock solution at the specified level, therefore demonstrating the precise parameters of the procedure. Using the test procedure as a guide, inject the solutions into the chromatographic system and compute the percentage of each individual contaminant, the confidence interval, and the %RSD. A similar approach was taken for Intermediate precision with different analysts, different HPLC system, different lot of columns, different day and with fresh individual preparations.

2.5.3 Linearity

All solutions were prepared by diluting impurity stock solutions to form an impurity mixture, which was then added to placebo solutions at different concentration levels, ranging from the limit of quantification (LOQ) to 150% of the designated test concentration. The area of the impurity peak (y-axis) was plotted against its predicted concentration in parts per million (ppm) to formulate a linear regression equation (x-axis). The residual sum of squares (R^2), slope, and intercept were derived from the regression line. The relative response factor was determined from the slopes of the impurity and the reference solution.

2.5.4 Accuracy

The accuracy study used a concentration range of 50% to 150% of the final test concentration. Test sample solutions were prepared in triplicate at each concentration level, ranging from 50% to 150% of the stated test concentration, with impurity stock solutions added at values between 0.5 ppm to 3 ppm. Calculated the recovery % of each impurity at every spike level.

2.5.5 Robustness

The robustness study was conducted to assess the effects of deliberate alterations in the chromatographic conditions, examining one factor at a time. The selected parameters for this study included flow rate (±0.1mL/min) and mobile phase pH (±0.2).

2.5.6 Limit of Detection and Limit of Quantitation

Determined the limits of detection (LOD) and quantitation (LOQ) values for Imatinib mesylate (analyte peak) and its impurities by introducing the lowest concentration level into a placebo solution to achieve a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ, respectively. Performed an evaluation of LOQ precision by formulating six test solutions through the introduction of impurities at the LOQ level into a placebo solution, meticulously prepared in accordance with the test method, and subsequently calculated the %RSD from the six spiked solutions.

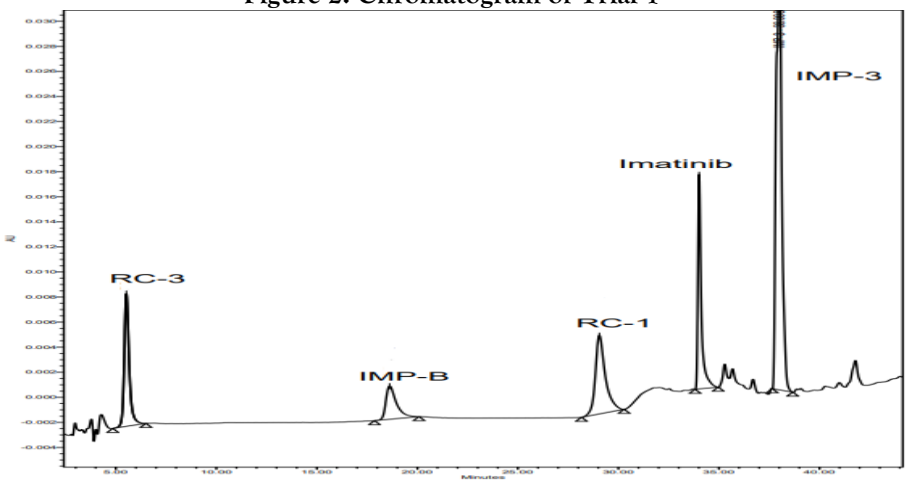
3. Results and Discussion

3.1 Method Development and Optimization

Prior to the development of methods, it is essential to ascertain a range of physicochemical parameters, including the pKa, log P, solubility, absorptivity, and the maximum wavelength of the drug. The pKa holds significance since the majority of pH-related alterations in retention transpire at pH levels that are within 1.5 units of the pKa value. Initial studies were carried out on each reaction solution separately, followed by an examination of a combination of these solutions, during which decomposition was observed. Commenced the separation process of the LC gradient program, by using 0.028M disodium hydrogen phosphate as mobile phase A and carefully adjusting its pH to 2.8 with Ortho Phosphoric acid.

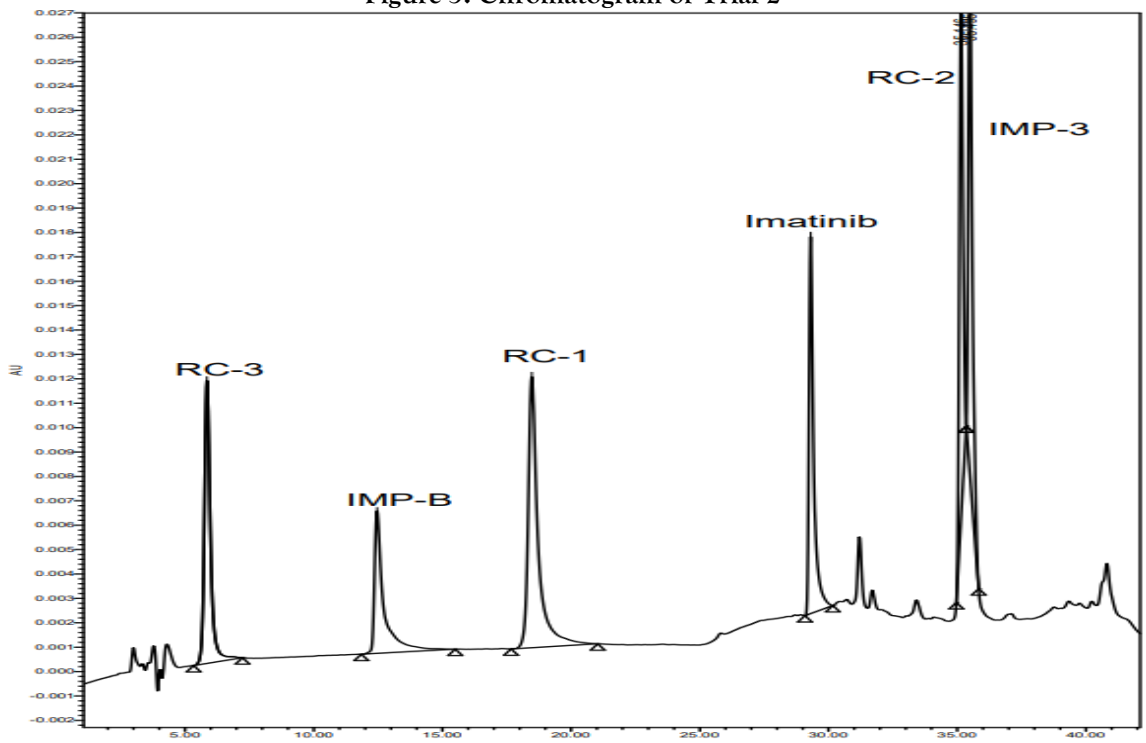
The acetonitrile utilized in mobile phase B exhibited a flow rate of 0.5 ml per minute, accompanied by a gradient program delineated as follows: Time/%MP B: 0/5, 10/5, 25/10, 35/80, 40/90, 42/5, 55/5. The injection volume was set at 20 µL, with the column maintained at a temperature of 25°C, that includes a Zodiac C18 column with dimensions of 150 x 4.6 and a particle size of 5µ. In the related compound analysis, compound 2 was not eluted during the initial run. (Trial 1: Figure 2)

Figure 2: Chromatogram of Trial 1



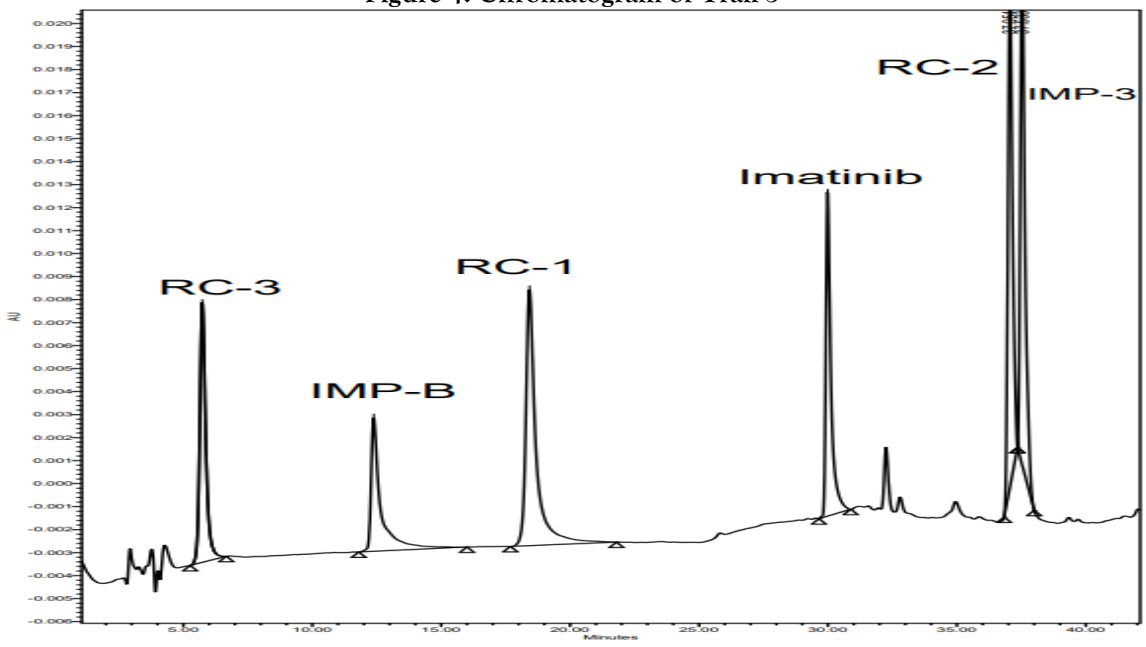
Consequently, the gradient was modified (Time/%MP B:0/5,20/15,35/80,37/80,40/5,45/5), indicating that RC 2 and IMP3 were merged. (Trial 2: Figure 3)

Figure 3: Chromatogram of Trial 2



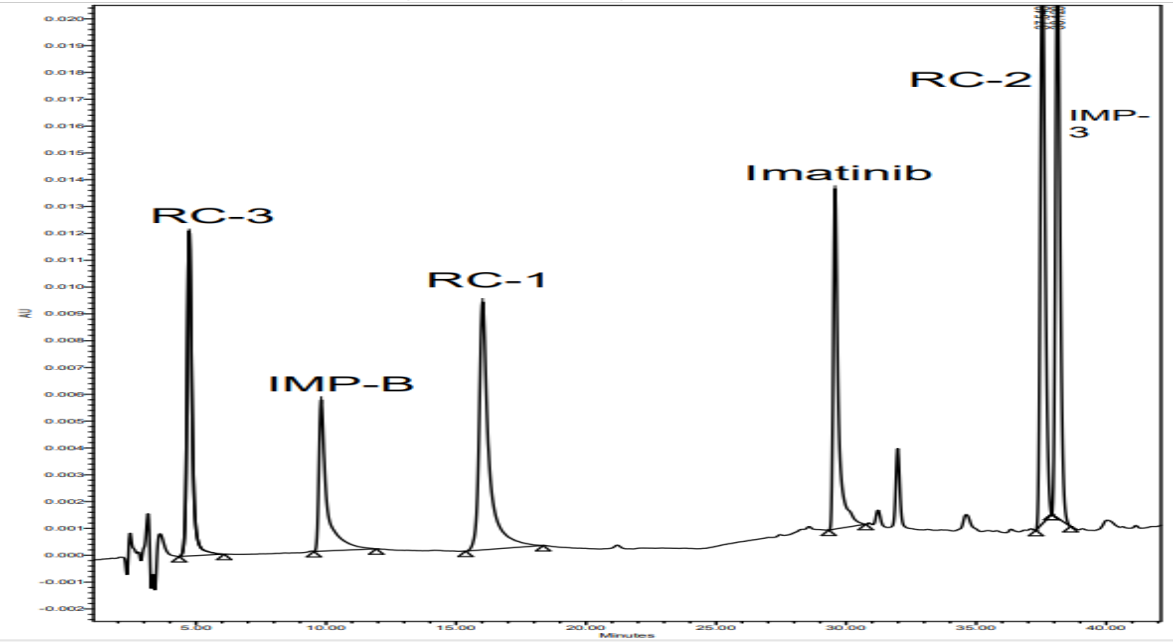
Further adjustments to the gradient (Time/%MP B:0/5,20/15,39/80,41/5,55/5) achieved separation, yet with a suboptimal resolution of approximately 1.48 between RC 2 and IMP 3. (Trial 3: Figure 4)

Figure 4: Chromatogram of Trail 3



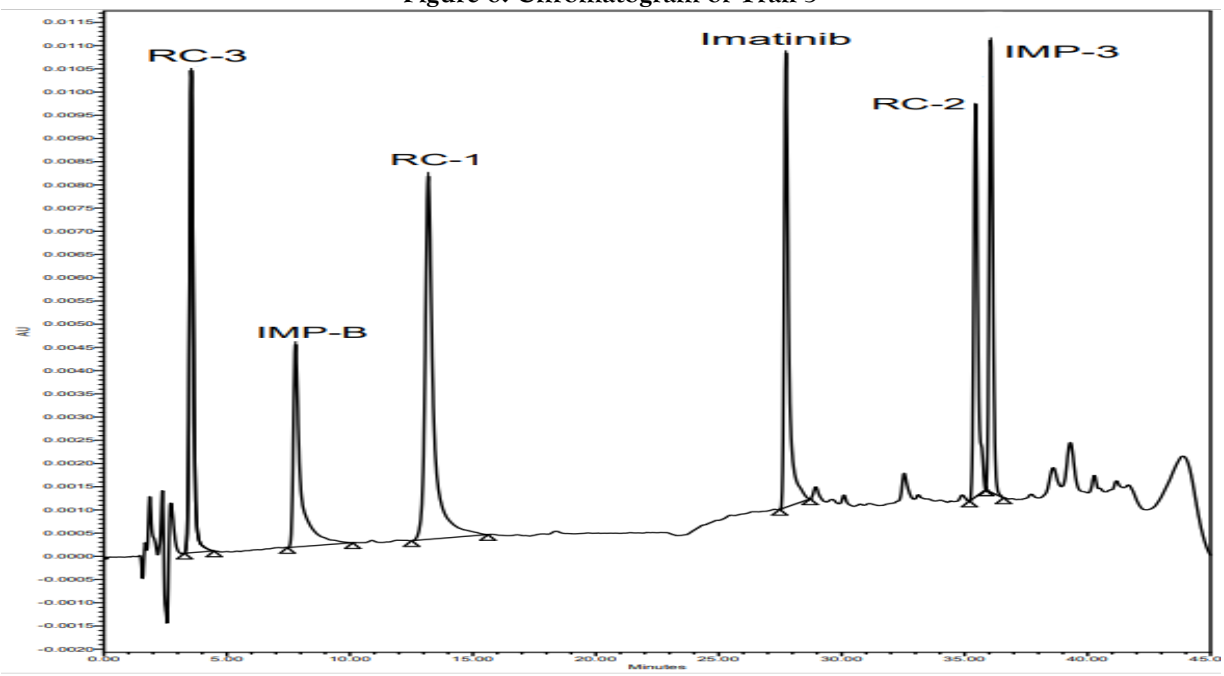
The column temperature was elevated from 25°C to 40°C to enhance resolution, while the flow rate was adjusted from 0.5 mL/min to 0.6 mL/min, resulted separation but poor resolution between RC 2 and IMP 3 about 1.74. (Trial 4: Figure 5)

Figure 5: Chromatogram of Trail 4



Further gradient modified as (Time/%MP B:0/5,20/15,39/65,41/5,45/5) with increase in flow rate from 0.6 mL/min to 0.8 mL/min, at 40°C column temperature, resulted good separation between RC 2 and IMP 3 about 2.10. (Trial 5: Figure 6)

Figure 6: Chromatogram of Trail 5



Additionally, the stabilization time during the gradient was shortened to 10 minutes after successive trials, leading to the finalizing of the method concluded as follows gradient program. (Table 6). Hence, finalized these optimized conditions for validation the conditions were given in Table 7.

Table 6 : Gradient programme for separation studies.

Time (min)	*Phosphate Buffer (%)*	*Acetonitrile (%)*	*Flow (mL/min)*
0	95	05	0.6
18	85	15	0.6
39	40	60	0.6
41	95	05	0.6
45	95	05	0.6

Table 7: Optimized chromatographic conditions

Diluents	0.028M disodium hydrogen phosphate (pH-2.8)
Mobile Phase	0.028M disodium hydrogen phosphate (pH-2.8): Acetonitrile
Flow rate	0.8 ml/min
Detection wavelength	240 nm
Run time	45 min
Temperature	25°C to 40°C
Volume of injection loop	50 µl
Retention time	27.2 min

3.2. Analytical Method Validation

3.2.1 Precision.

The individual spiked impurities in the test solution, derived from method precision, were determined to be at the specification level of 0.2%. The %RSD values for RC-1, RC-2, RC-3, IMP-3 and IMP-B were recorded as 0.7%, 0.4%, 0.5%, 2.3% and 0.7%, respectively. The %RSD results indicate a commendable consistency among the individual preparations, confirming the method's precision. The computed 95% confidence intervals pertaining to Method precision are presented in Table-8.

Table 8: Precision results

Impurity Name	**Obtained %RSD**	**Acceptance %RSD**
RC-1	0.7%	NMT 15%
RC-2	0.4%	NMT 15%
RC-3	0.5%	NMT 15%
IMP-B	2.3%	NMT 15%
IMP-3	0.7%	NMT 15%

3.2.2 Linearity.

The linearity of the detector response was established for all five impurities and Imatinib mesylate across a range from the limit of quantitation (LOQ) to 150%, specifically at LOQ, 50%, 75%, 100%, 125%, and 150% of the test concentration levels. The calibration curve was constructed by plotting the concentration of linearity solutions on the x-axis (in ppm) against the area responses on the y-axis (AU) through linear regression analysis. The areas of the impurity peaks demonstrated a linear relationship across the concentration range, exhibiting correlation coefficients (R^2) > 0.99 [Table-9]. The established linear correlation between the concentration of solutions and their corresponding area responses indicates that the current methodology is capable of quantifying impurities within this defined linear range. The calibration curves were provided in Figure 7 to 12.

Table 9: Linearity

Impurity Name	**Correlation Coefficient**	**RRF**
RC-1	0.999	1.61
RC-2	0.995	1.15
RC-3	0.993	1.04
IMP-3	0.994	1.26
IMP-B	0.990	0.75
Imatinib	0.990	NA

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
https://www.theaspd.com/ijes.php

Figure 7: Linearity plot of RC-1

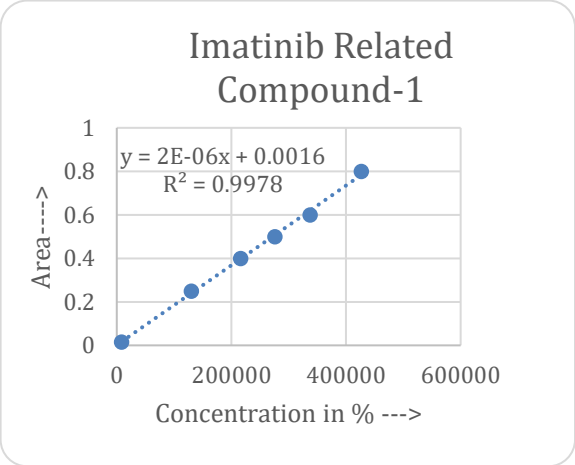


Figure 8: Linearity plot of RC-2

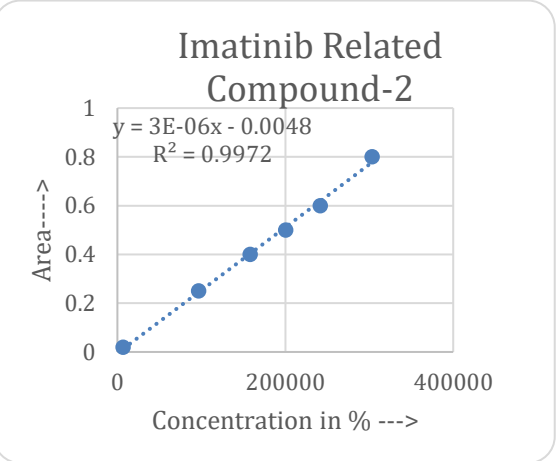


Figure 9: Linearity plot of RC-3

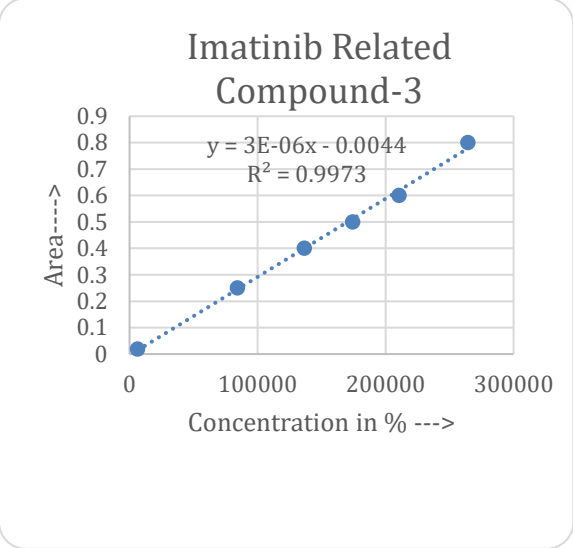


Figure 10: Linearity plot of IMP-3

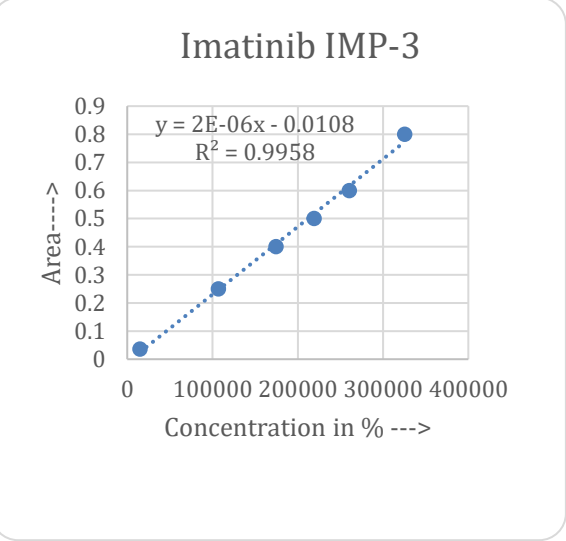


Figure 11: Linearity plot of IMP-B

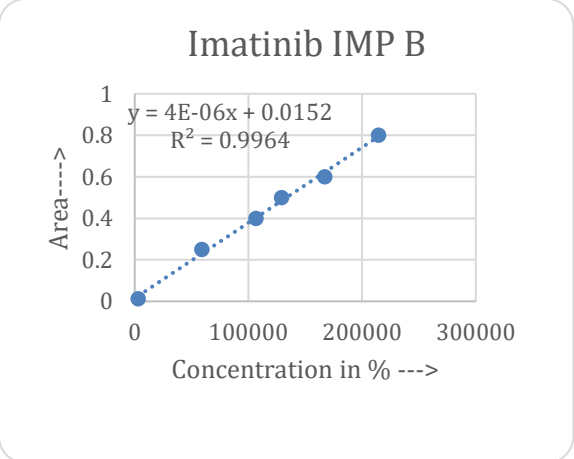
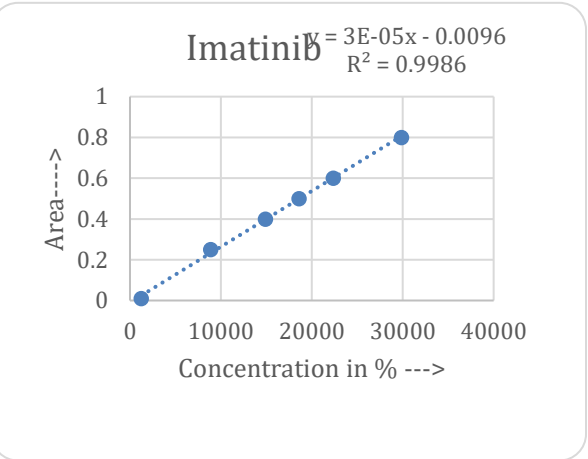


Figure 12: Linearity plot of Imatinib



3.2.3 Sensitivity.

Limit of detection, Limit of quantification values are reported in Table 10. The %RSD for peak areas of impurities and Imatinib are with 5%.

Table 10: Sensitivity results

Impurity Name	LOD S/N	LOQ S/N	LOQ %RSD	LOQ Accuracy
RC-1	3.0	9.8	8.1	109.2
RC-2	3.3	10.2	5.6	106.1
RC-3	3.1	10.0	3.1	103.2
IMP-3	3.0	9.9	7.8	89.2
IMP-B	3.2	10.0	9.1	91.5
Imatinib	3.0	9.0	4.2	102.1

3.2.4 Accuracy (Recovery).

The recovery study was conducted at 50%,100% and 150% levels by spiking impurities mixture at 0.2% level on test sample solution with respect to targeted test concentration. The average recovery obtained for all three impurities at three distinct levels ranged from 90% to 110%. Table 11 illustrate the recovery results.

Table 11: Accuracy

Impurity Name	Preparation-01	Preparation-02	Preparation-03
RC-1	106.18%	106.64%	107.24%
RC-2	108.53%	108.30%	108.73%
RC-3	97.33%	98.37%	98.25%
IMP-3	113.21%	112.70%	112.49%
IMP-B	112.20%	110.04%	106.86%

3.2.5 Robustness.

Results of robustness in flowrate and mobile phase pH given in Table 12.

Table 12: Robustness

Impurity name	As such method RRT	Low flow method RRT	High flow method RRT	Low column temp. RRT	High Column. Temp RRT
RC-1	0.534	0.579	0.497	0.542	0.529
RC-2	1.325	1.310	1.336	1.329	1.317
RC-3	0.167	0.191	0.151	0.171	0.167
IMP-3	1.351	1.335	1.364	1.357	1.341
IMP-B	0.338	0.374	0.309	0.352	0.33

3.2.6 Stress Studies.

HPLC studies on Imatinib Mesylate under different stress conditions suggested the following degradation behavior. The drug was found to be susceptible to acid hydrolysis (2N HCl for 1hour), with 24.3% degradation when heated at 60 °C, but was stable in neutral hydrolysis (water, 1hour) The drug was found to be susceptible to peroxide degradation (H_2O_2 for 1hour), with 27.3% degradation .No Significant basic degradation was observed when the sample was kept in 2N NaOH at 60° for 1hour. No Significant thermal degradation was also observed when the sample was kept in at 120° for 3 h (Table 13). The individual chromatograms for stressed sample were provided from Figure 13 to 17 and peak purity plots were provide from Figure 18 to 22.

Table 13: Forced Degradation study results and Mass Balance of Stressed samples

Forced Degradation Study	ASSAY	%Degradation	Mass balance
Acid Stress	76.0	24.3	101.31
Base Stress	99.2	0.2	100.40
Peroxide Stress	72.3	27.3	100.61
Thermal Stress	99.6	0.1	100.71
Water Stress	99.0	0.1	100.10

Figure 13: Chromatogram of degradation products and active pharmaceutical ingredient of Imatinib when stressed with acid.

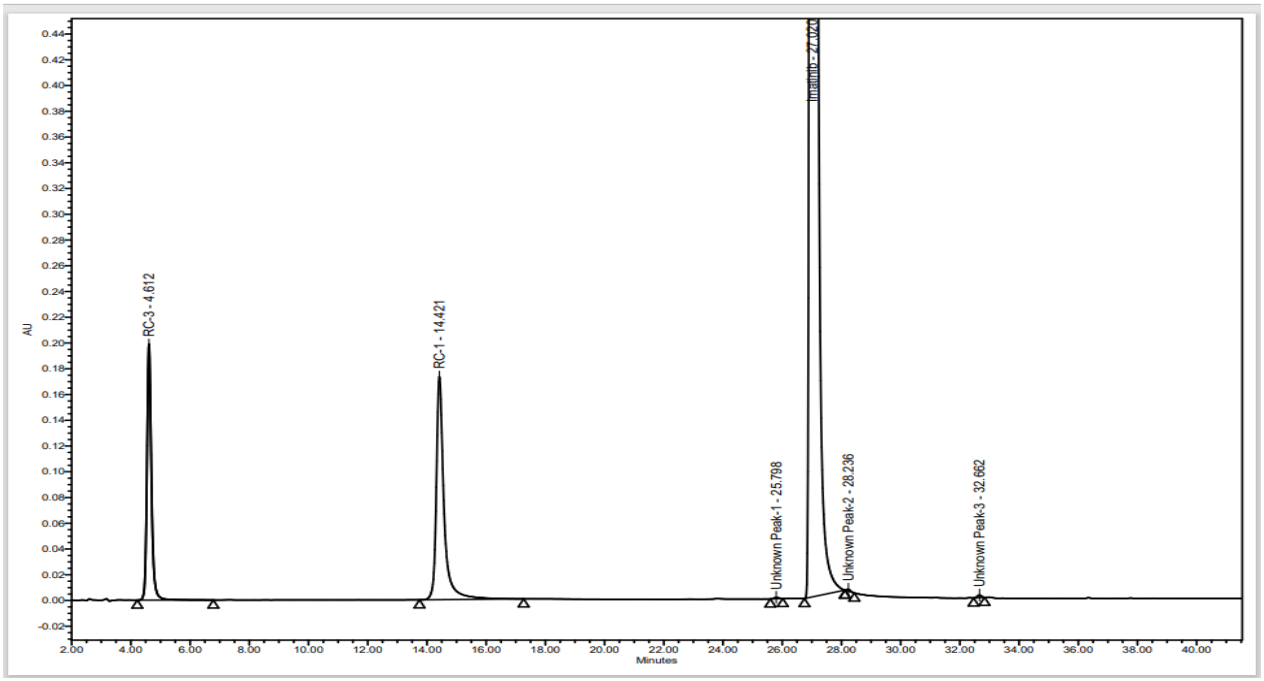


Figure 14: Chromatogram of degradation products and active pharmaceutical ingredient of Imatinib when stressed with water.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
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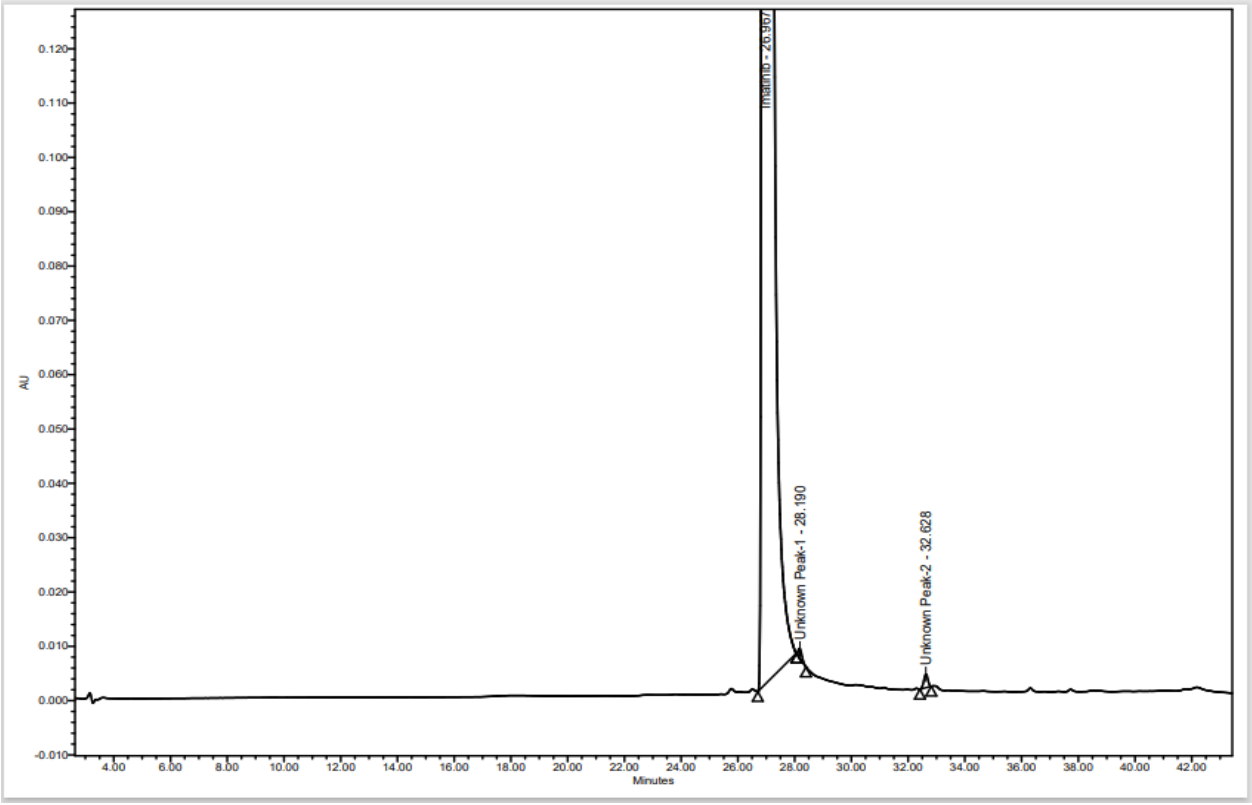

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
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Figure 15: Chromatogram of degradation products and active pharmaceutical ingredient of Imatinib when stressed with 2N NaOH.

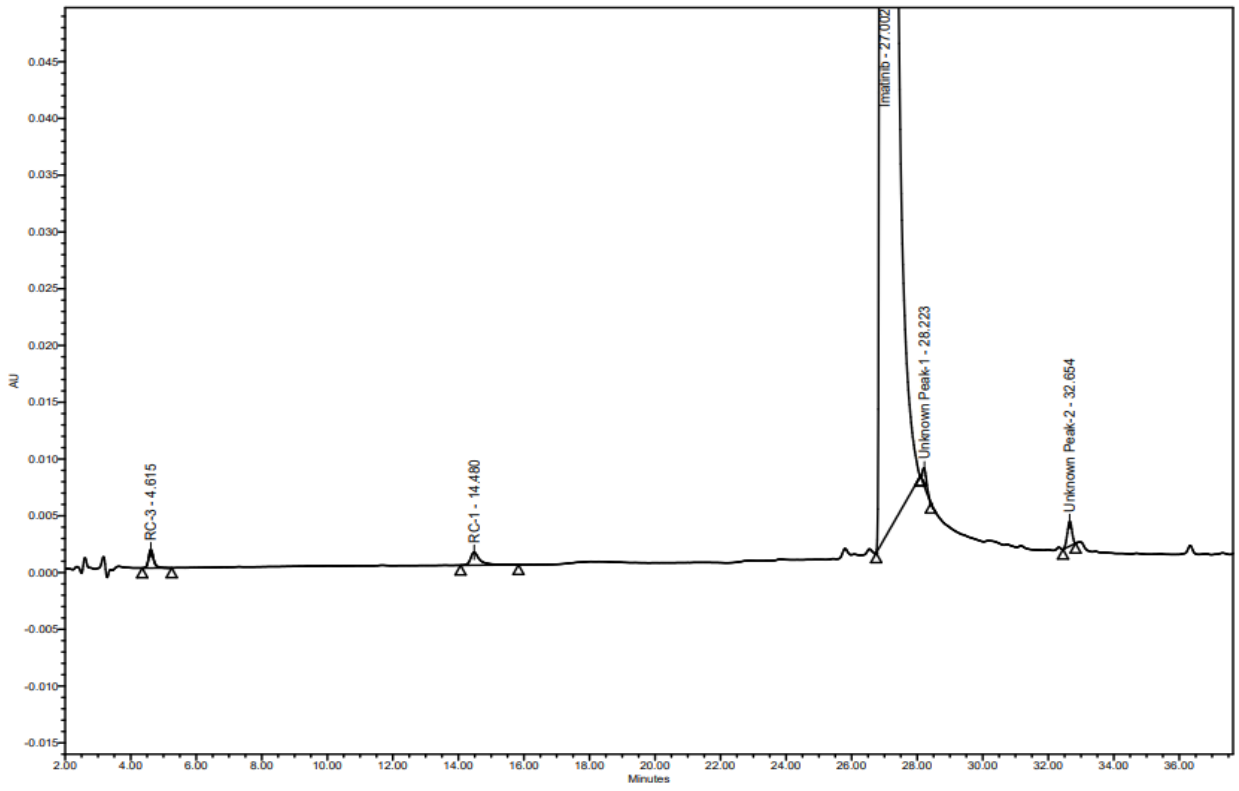

Figure 16: Chromatogram of degradation products and active pharmaceutical ingredient of Imatinib when stressed with Heat.

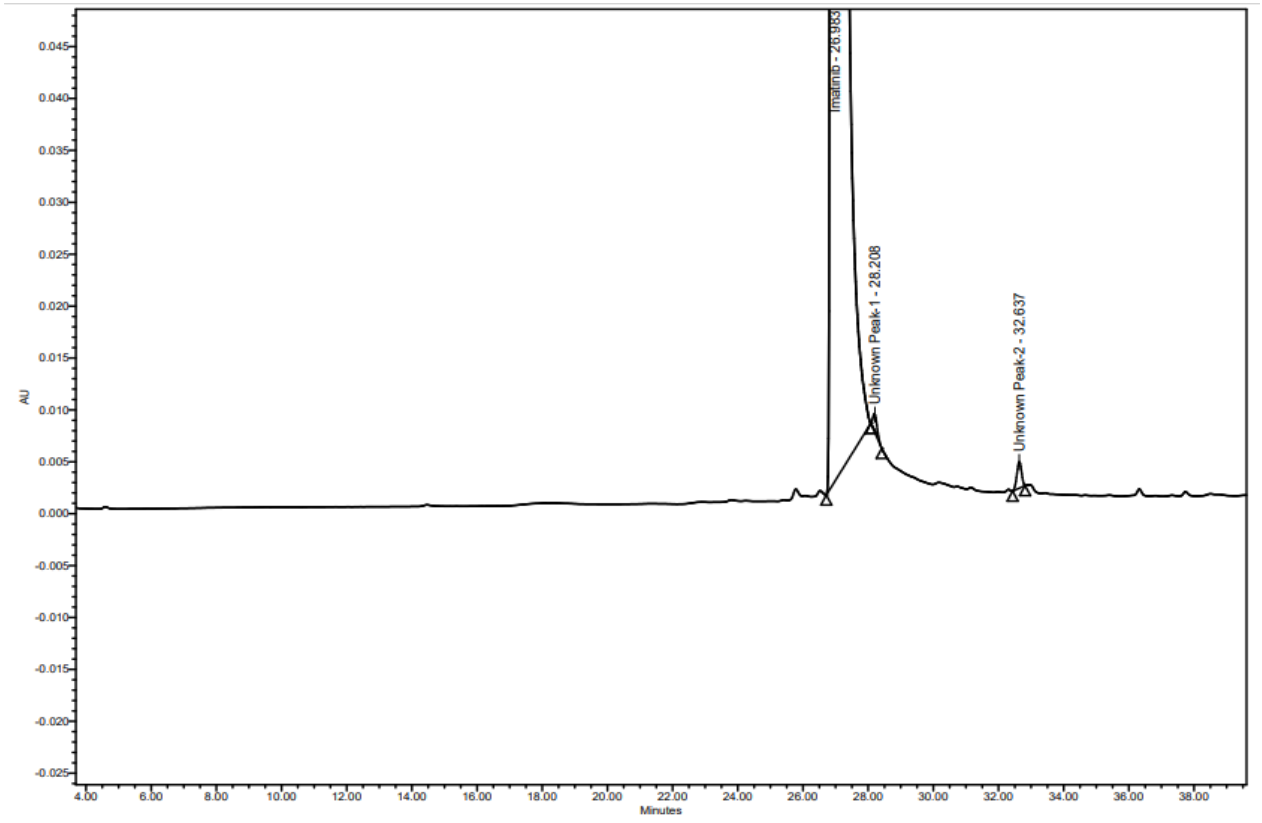

Figure 17: Chromatogram of degradation products and active pharmaceutical ingredient of Imatinib when stressed with peroxide

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
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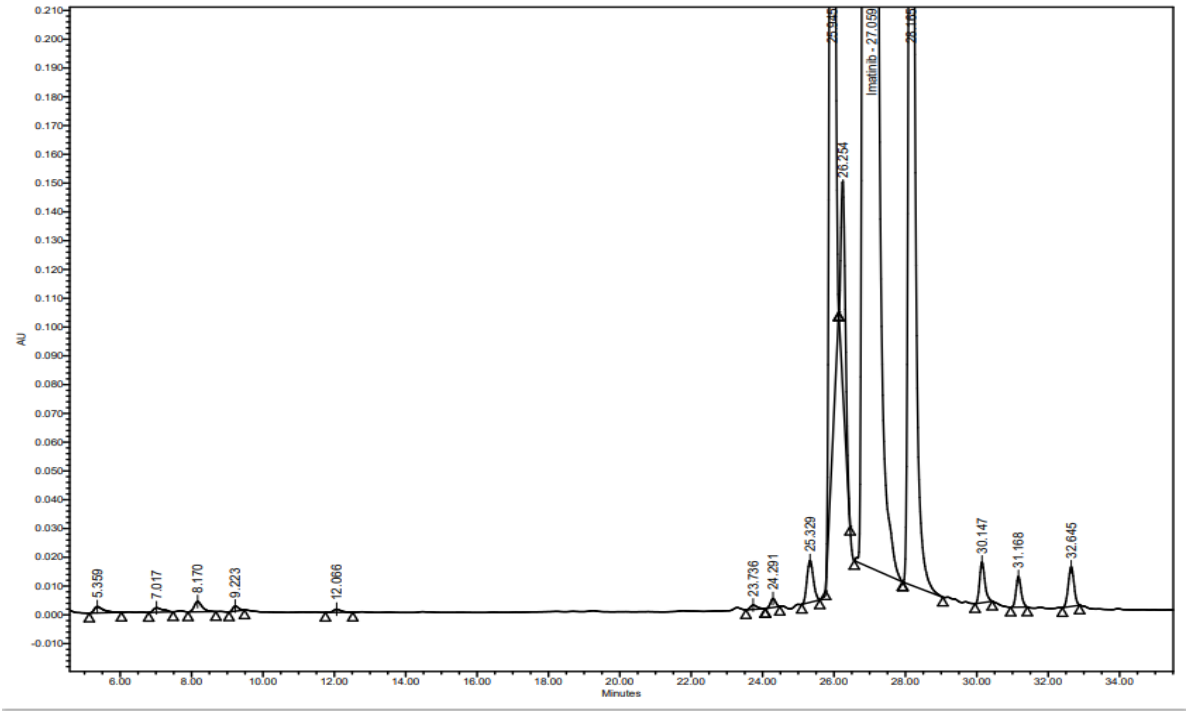


Figure 18: Peak purity plot in Acid Hydrolysis stress sample

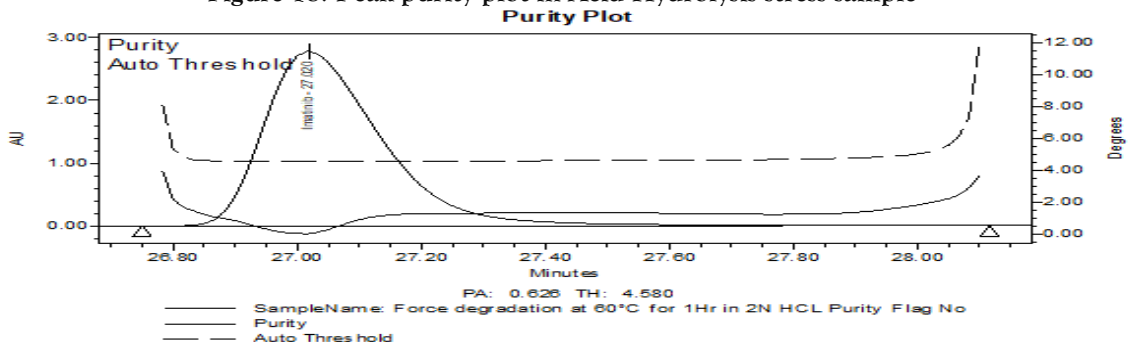


Figure 19: Peak purity plot in Base Hydrolysis stress sample

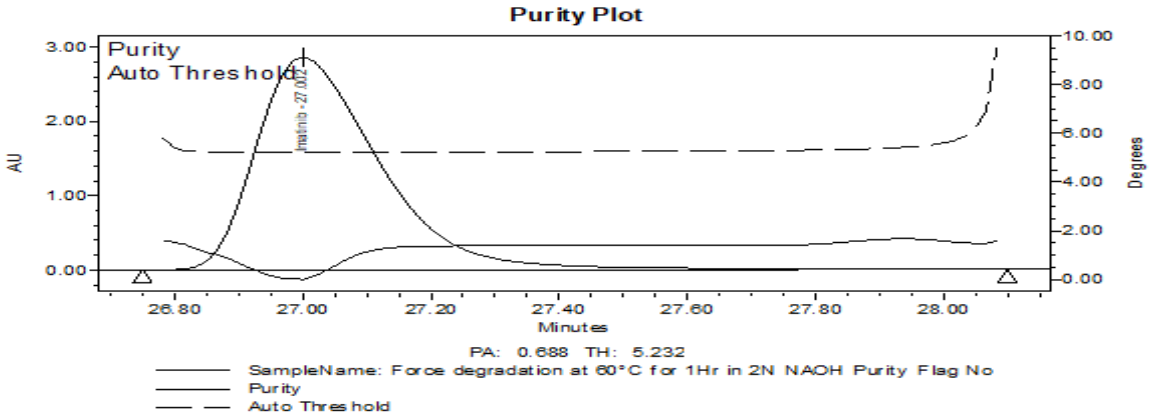


Figure 20 : Peak purity plot in Thermal stress sample

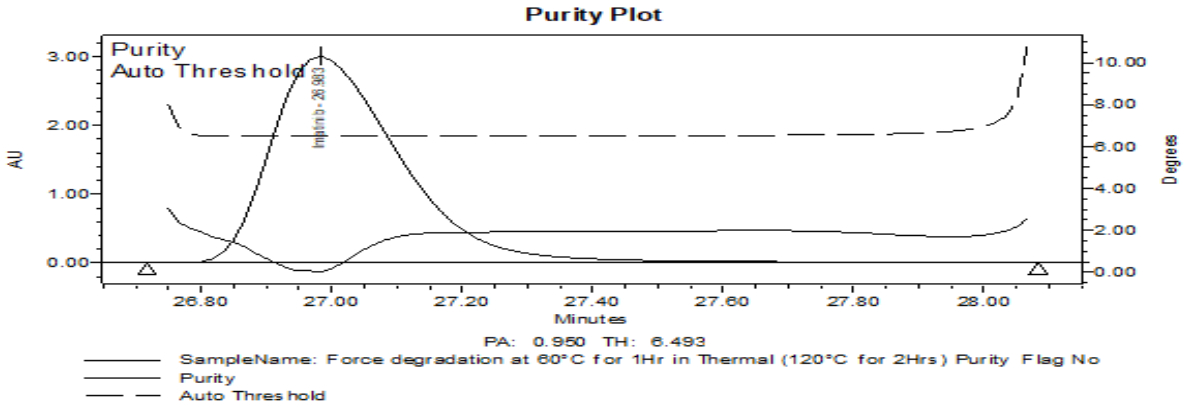


Figure 21: Peak purity plot in Water stress sample

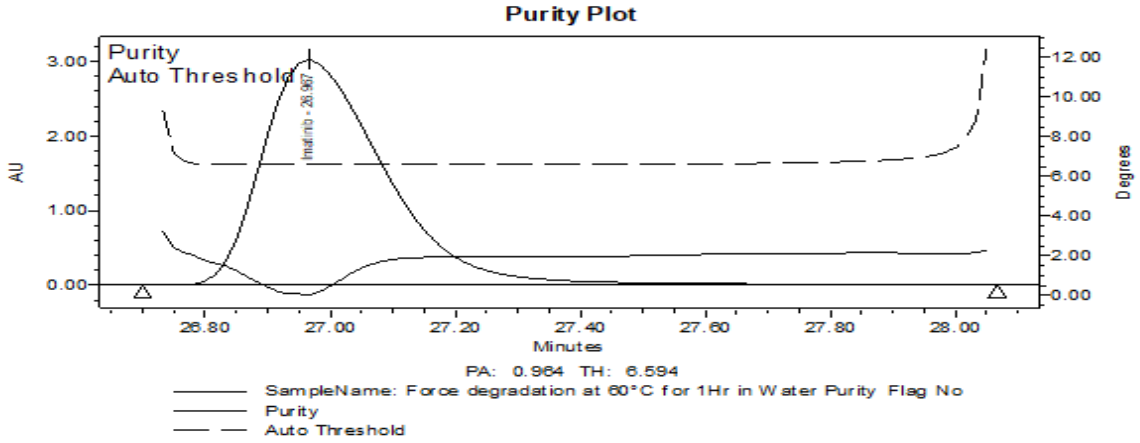
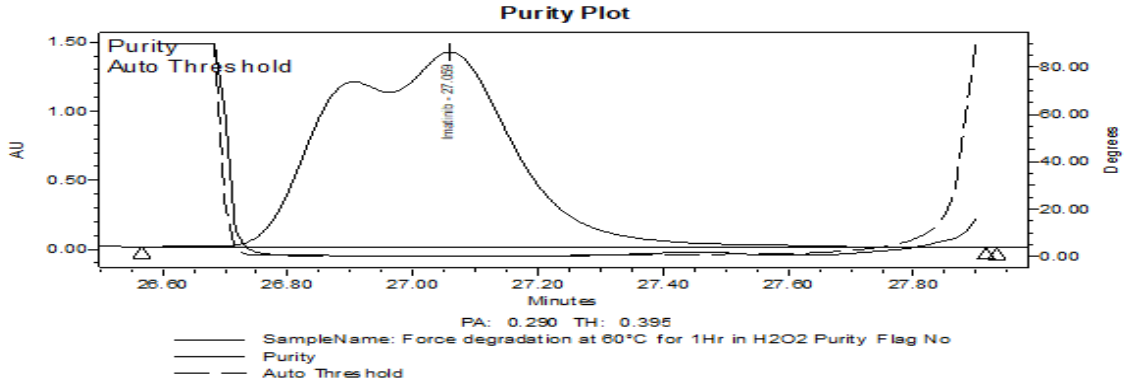


Figure 22: Peak purity plot in peroxide stress sample



4.0 Conclusion

The RP-HPLC method allows for precise separation of Imatinib mesylate impurities, ensuring that the product is free from contaminants. The proposed optimized method produces good sharp peak and with good resolution between the drug and its impurities. The method provides the high peak purity which implies the separation and purification of impurities from drug. This method provides the advantage of low run time for both impurities and drug elution which allows time efficient analysis. Method is highly sensitive and selective, capable of detecting even trace amounts of impurities which enables stringent quality control. There is allowable variation in flow rate, temperature, pH and mobile phase composition, which indicate that the method is robust. The low relative SD for the spiked impurities of the test preparation shows that the proposed method is rugged. This study shows that the drug is highly sensitive to degradation with acid and peroxide stress but stable in dry heat, basic and Hydrolysis conditions. This method allows separation of all degradation products formed under a variety of conditions. The method is stability indicating and appropriate for intended use to analysis at quality control labs.

6.Consent for publication:
The authors declare no conflict of interest.

7.Competing interests:
The authors declare that they have no competing interests.

8. Acknowledgements:
The author wishes to acknowledge and grateful to Lucid Laboratories, Hyderabad, India, for providing research facility to carry out the research. Author thanks full to Research Co- Supervisor Mallikharjuna Rao Bandlamudi for continuous support in arranging facilities and guidance on research work. And thank full to Research supervisor Dr. V Srinivasa Murthy and K. Anand Solomon, Department of Chemistry, School of Engineering, Devarakaggalahalli, Harohalli for his guidance on research.

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