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METHOD DEVELOPMENT, VALIDATION AND ESTIMATION OF SINGLE SYNTHETIC DRUG TUCATINIB BY RP-HPLC GREEN ANALYSIS IN API FORM AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

An analytical, simple, accurate, robust, specific, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Tucatinib in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved on a Hypersil ODS C18 column (2.1 mm × 100 mm, 5 µm) using an optimized eco-friendly mobile phase consisting of ethanol and water (65:35% v/v) at a flow rate of 0.7 mL/min, with detection carried out at 260 nm. The retention time of Tucatinib was found to be 2.3 ± 0.02 min, indicating rapid analysis with reduced solvent consumption. The method exhibited excellent linearity over the concentration range of 24–120 µg/mL with a correlation coefficient (r) of 0.998. The precision of the method was confirmed with %RSD values less than 2.0%. The method was validated in accordance with ICH guidelines for accuracy, precision, linearity, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ). The percentage recovery was found within the acceptable range of 98–102%, confirming the accuracy of the method. The developed method was successfully applied for the quantitative estimation of Tucatinib in pharmaceutical tablet formulations, yielding assay results of 99.7%. In addition to analytical validation, the method was evaluated for its environmental sustainability using green analytical chemistry assessment tools. The Analytical Eco-scale score was found to be 91, indicating excellent greenness. The AGREE score of 0.82 demonstrated high compliance with the principles of green analytical chemistry, while the GAPI assessment revealed predominantly green zones with minimal environmental impact. The replacement of conventional toxic solvents such as acetonitrile with ethanol significantly enhanced the eco-friendly profile of the method. In conclusion, the developed RP-HPLC method is not only reliable and efficient but also environmentally sustainable, making it highly suitable for routine quality control analysis in pharmaceutical industries.

Key Words: *Tucatinib, RP-HPLC, Method Development, Validation, Green Analytical Chemistry, AGREE, GAPI, Eco-scale.*

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INTRODUCTION:

Tucatinib is a kinase inhibitor used to treat certain types of unresectable/metastatic HER-2 positive breast cancer. Tucatinib is a kinase inhibitor drug used with Trastuzumab and Capecitabine in the treatment of unresectable or metastatic HER-2 positive breast cancer [1]. It was developed by Seattle Genetics and approved by the FDA on April 17, 2020. Tucatinib (Tukysa) is a targeted tyrosine kinase inhibitor used to treat HER2-positive breast and colorectal cancers by blocking abnormal protein signals that cause cancer growth. It is primarily used in combination with Trastuzumab and Capecitabine for advanced, metastatic, or unresectable cancers, and is noted for its ability to treat tumors that have spread to the brain. Tucatinib is a Kinase Inhibitor. The mechanism of action of Tucatinib is as a Tyrosine Kinase Inhibitor, and Cytochrome P450 3A Inhibitor, and P-Glycoprotein Inhibitor, and Cytochrome P450 2C8 Inhibitor [2]. Tucatinib, sold under the brand name Tukysa, is an anticancer medication used for the treatment of HER2-positive breast cancer. It is a small molecule inhibitor of HER2. It was developed by Array BioPharma and licensed to Cascadian Therapeutics (formerly Oncothyreon, subsequently part of Seattle Genetics). Common side effects include Diarrhea, palmar-plantar erythrodysesthesia (burning or tingling discomfort in the hands and feet), nausea, fatigue, hepatotoxicity (liver damage), vomiting, stomatitis (inflammation of the mouth and lips), decreased appetite, abdominal pain, headache, anaemia and rash. Tucatinib may cause harm to a developing foetus or baby [3]. The IUPAC Name of Tucatinib is 6-N-(4, 4-dimethyl-5H-1, 3-oxazol-2-yl)-4-N-[3-methyl-4-([1, 2, 4] triazolo [1, 5-a] pyridin-7-yloxy) phenyl] quinazoline-4, 6-diamine. The Chemical Structure of Tucatinib is shown in follows

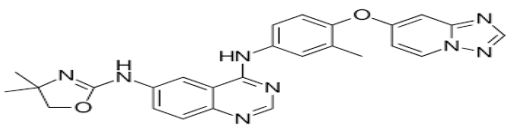


Fig-1: Chemical Structure of Tucatinib

A survey of literature reveals that few analytical methods are available for the drugs like Tucatinib [21-22]. The existing phyisico-chemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for estimation Tucatinib in bulk and marketed pharmaceutical dosage forms adopting different available analytical techniques like HPLC. According to the literature survey it was found that few analytical methods on HPLC-MS/MS, HPLC were reported for rizatriptan benzoate.

The objective of the proposed methods is to develop simple and accurate methods for the estimation of Tucatinib in bulk and pharmaceutical dosage forms by HPLC. Method development by RP-HPLC method for Tucatinib involves the development of suitable mobile phase, optimization of the chromatographic conditions, selection of suitable detection wavelength, and preparation of standard calibration curve of Tucatinib, assay of pure mixed standards and formulation and finally validation of the developed method.

MATERIALS AND METHODS:

Instruments and Chemicals

Instruments: The chromatographic analysis was performed using a Waters Alliance 2695 HPLC system equipped with a 996 Photodiode Array (PDA) detector and Empower 2 software for data acquisition and processing. The pH measurements were carried out using a Lab India pH meter. Analytical weighing was performed using a Sartorius electronic balance. Volumetric flasks, pipettes, burettes, and beakers used throughout the study were of Borosil make. Sample preparation and degassing of solutions were carried out using a Labman digital ultrasonic Sonicator.

Chemicals: The pure Tucatinib reference standard was procured from Synpharma Research Laboratories, Hyderabad. HPLC-grade water and methanol were obtained from Lichrosolv (Merck), while HPLC-grade acetonitrile was purchased from Merck. All chemicals and reagents used in the study were of analytical or HPLC grade and were used without further purification.

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.72ml of the above Tucatinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [6].

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and Water in proportion 65:35% v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like ODS column, Xterra, and X Bridge C18 column. Hypersil ODS C18 (4.6mm x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow [4].

Preparation of Mobile Phase:

Accurately measured 650 ml (65%) of HPLC Acetonitrile and 350 ml of HPLC Water (35%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.72ml of the above Tucatinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [5].

Specificity Study of Drug:

Preparation of Standard Solution:

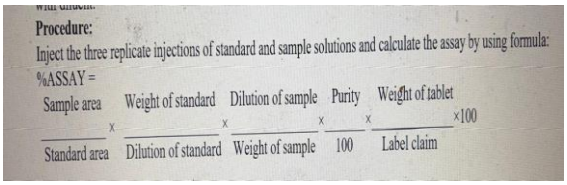
Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.72ml of the above Tucatinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Tucatinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.72ml of Tucatinib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.


Procedure:
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:
%ASSAY =
$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (24ppm of Tucatinib):

Pipette out 0.24ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – II (48ppm of Tucatinib):

Pipette out 0.48ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – III (72ppm of Tucatinib):

Pipette out 0.72ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – IV (96ppm of Tucatinib):

Pipette out 0.96ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – V (120ppm of Tucatinib):

Pipette out 1.2ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [7].

Precision

Repeatability

Preparation of Tucatinib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.72 ml of the above Tucatinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions [8].

Procedure:

Day 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.36ml of the above Tucatinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [9].

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.72ml of the above Tucatinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.08ml of the above Tucatinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Tucatinib and calculate the individual recovery and mean recovery values [10].

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Tucatinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.72ml of the above Tucatinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.7 ml/min and 0.9 ml/min instead of 0.8ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded [11].

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio and 70:30, 60:40 instead of 65:35, remaining conditions are same.10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Analytical Method Development:

The optimized chromatographic conditions for the developed RP-HPLC method were established using a Hypersil C18 ODS column (2.1 mm × 100 mm, 5 µm particle size) as the stationary phase. Efficient chromatographic separation was achieved with a mobile phase consisting of Ethanol and Water in the ratio of 65:35 (% v/v), delivered at a flow rate of 0.7 mL/min under isocratic conditions. The analysis was performed at ambient temperature (25–30°C), and the eluents were monitored using a UV detector set at 260 nm. A 10 µL injection volume was employed for all chromatographic runs. Under these optimized conditions, satisfactory peak resolution, symmetry, and reproducibility were obtained within a total run time of 6 minutes, making the method suitable for routine quantitative analysis and stability studies [12]. The optimized chromatogram was showed in following fig-2.

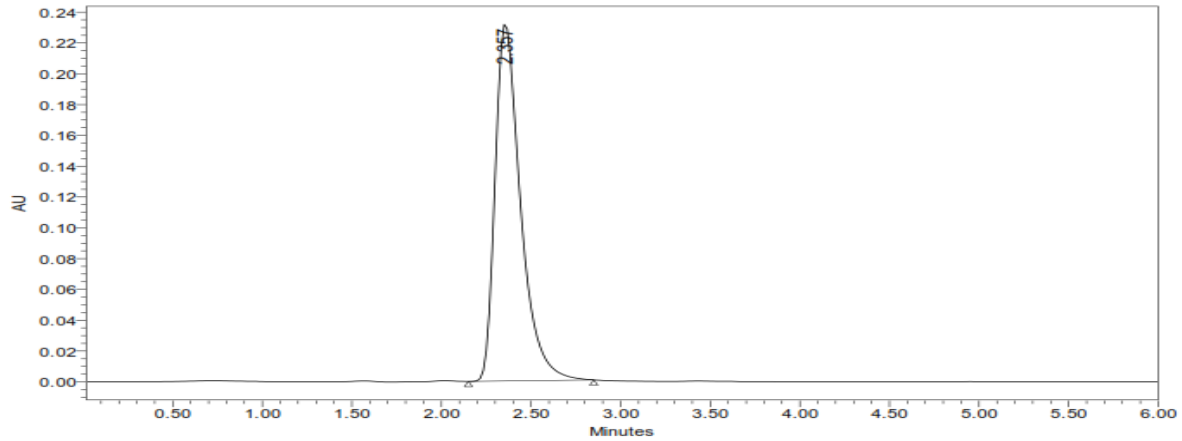


Fig-2: Optimized Chromatographic Condition

Validation of Analytical Method:

The developed analytical method was validated in accordance with the International Council for Harmonisation (ICH) Q2 (R1) and Q2 (R2) guidelines [6]. The validation study included assessment of specificity, linearity, accuracy, and precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness, robustness, and to demonstrate the suitability of the method for its intended analytical application [13].

System Suitability:

It can be defined as tests to ensure that the method can generate acceptable accuracy and precision results. The USP defines parameters that can be used to determine the system's suitability before analysis. These parameters include plate no. (n), tailing factor, k and/or α, resolution (Rs), and relative standard deviation (RSD) of peak height or peak area or repetitive injections. The results were shown in table-1.

Table-1: Results of System Suitability for Tucatinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tucatinib	2.317	2274631	239458	5728	1.2
2	Tucatinib	2.302	2284721	239582	5093	1.2
3	Tucatinib	2.323	2238127	236493	5391	1.2
4	Tucatinib	2.343	2259349	249482	6139	1.2
5	Tucatinib	2.321	2204850	239452	5281	1.2
Mean			2252336			
Std. Dev.			31827.08			
% RSD			1.41307			

Specificity:

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Tucatinib in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Observation: The % purity of Tucatinib in pharmaceutical dosage form was found to be 99.7%.

Linearity: The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurement at several analyte

concentrations. The data are then processed using a linear least-squares regression. The resulting plot slope, intercept, and correlation coefficient provide the desired information on linearity. The numerical value of the slope and intercept will depend on the response measured, but intercepts greater than 2% (relative to the target level response) are typically expected with well-designed HPLC methods for major component analysis [14]. A linearity correlation coefficient above 0.999 is acceptable for most methods.

Table-2: Linearity Data of Tucatinib

Concentration µg/ml	Average Peak Area
24	791554
48	1647073
72	2283804
96	3058339
120	3839630

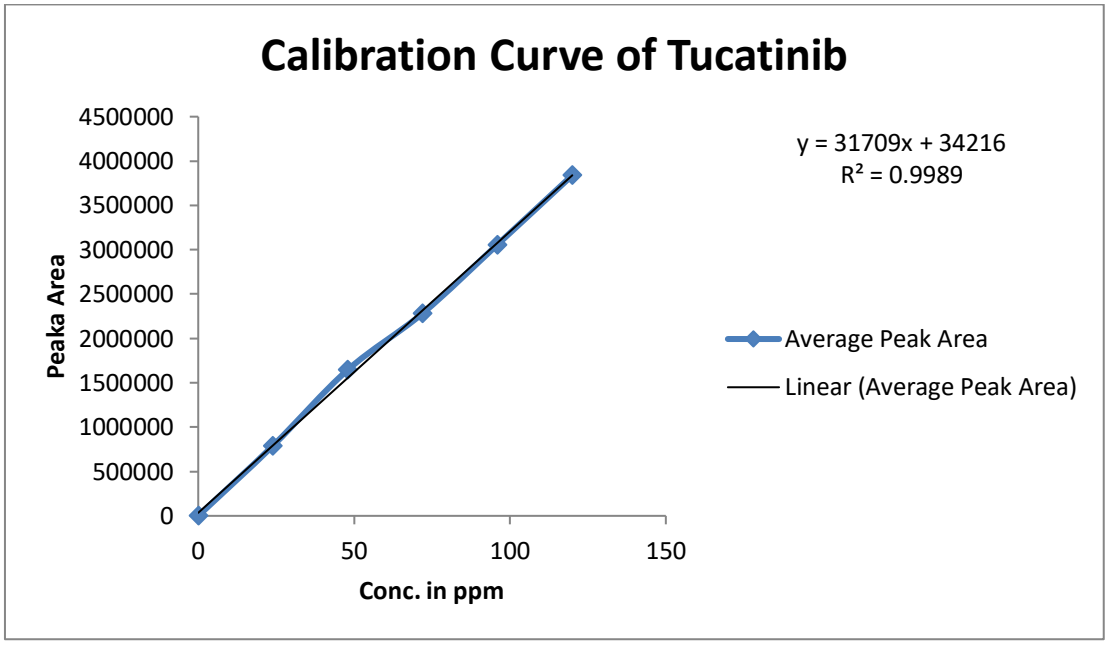


Fig-3: Calibration Curve of Tucatinib

Precision: The method's precision was established by analyzing the compound based on intra-day and inter-day analysis. Precision was calculated by the relative standard deviation (% RSD).

Repeatability: Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD [15].

Table-3: Results of Repeatability for Tucatinib

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tucatinib	2.356	2259464	245362	5938	1.2
2	Tucatinib	2.356	2275915	248293	5827	1.2
3	Tucatinib	2.357	2282117	240795	5032	1.2
4	Tucatinib	2.358	2278675	230139	5978	1.2
5	Tucatinib	2.359	2282448	249605	6183	1.2
Mean			2275724			
Std. Dev			9476.485			
%RSD			0.416416			

Intermediate Precision:

Analyst-1:

Table-4: Results of Intermediate Precision for Tucatinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Tucatinib	2.380	2236184	202188	5472	1.2
2	Tucatinib	2.383	2238020	201837	6193	1.2
3	Tucatinib	2.385	2239352	201273	5980	1.2
4	Tucatinib	2.385	2242466	203923	7163	1.2
5	Tucatinib	2.389	2244692	202938	6182	1.2
6	Tucatinib	2.389	2247654	201982	7684	1.2
Mean			2241395			
Std. Dev.			4333.851			
% RSD			0.193355			

Analyst-2:

Table-5: Results of Intermediate Precision Analyst 2 for Tucatinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Tucatinib	2.380	2236184	217363	5928	1.2
2	Tucatinib	2.383	2238020	218467	6183	1.2
3	Tucatinib	2.385	2239352	218346	5927	1.2
4	Tucatinib	2.385	2242466	221736	5163	1.2
5	Tucatinib	2.389	2244692	228361	4827	1.2
6	Tucatinib	2.346	2263431	217553	5019	1.2
Mean			2244024			
Std. Dev.			9988.458			
% RSD			0.445114			

Accuracy: The accuracy of Tucatinib was performed by calculating recovery studies of the test sample at three different concentration levels (50%, 100%, and 150%) by the standard addition method. At each level, three replicates were injected into a chromatographic system. The mean percentage recovery for Tucatinib was found within a limit of 98–102%, and from percentage recovery results, it was found that the developed method is accurate [16]. The percentage recovery results are tabulated in Tables 6.

Table-6: The Accuracy Results for Tucatinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1172485	36	35.8	99.4	99.5%
100%	2314753	72	71.6	99.4	
150%	3480210	108	107.9	99.9	

Limit of Detection for Tucatinib:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [17].

$$LOD = 3.3 \times \sigma / s$$

Where,

σ = Standard deviation of the response and S = Slope of the calibration curve

Result: 5.5µg/ml

Quantitation Limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10\times\sigma/S$$

Where,

σ = Standard deviation of the response and S = Slope of the calibration curve

Result: 16.7µg/ml

Robustness: The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tucatinib. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Tucatinib were injected by changing the conditions of chromatography [18]. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-7: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 0.8mL/min	3119086	2.379	5837	1.2
Less Flow rate of 0.7mL/min	2640811	2.763	5361	1.2
More Flow rate of 0.9mL/min	2640354	2.234	5231	1.2
Less organic phase	2640758	2.765	4503	1.5
More organic phase	2640125	2.236	4491	1.5

Green Analytical Assessment (Green Analysis):

The environmental sustainability of the developed RP-HPLC method for the determination of Tucatinib was systematically evaluated using three widely accepted Green Analytical Chemistry (GAC) assessment tools, namely the Analytical Eco-Scale; AGREE metric, and Green Analytical Procedure Index (GAPI). The chromatographic method employed an environmentally favorable mobile phase consisting of ethanol and water (65:35, v/v), thereby eliminating the use of hazardous organic solvents such as acetonitrile and methanol. The method was further characterized by a low flow rate (0.7 mL min^{-1}), short analysis time (6 min), and operation at ambient temperature, all of which contributed to reduced solvent consumption, lower energy requirements, and minimized waste generation [19].

1. AGREE Metric Evaluation

Fig-4: AGREE Graph

2. Analytical Eco-Scale Evaluation

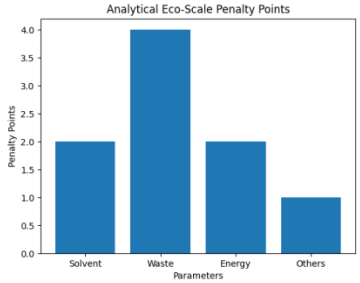


Fig-5: Eco-Scale Bar Graph

3. GAPI Assessment

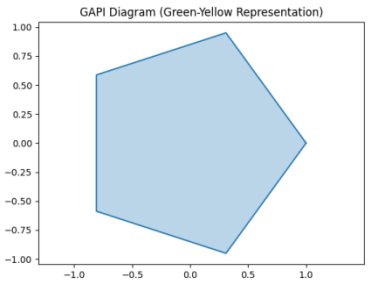


Fig-6: GAPI Diagram

The Analytical Eco-Scale evaluation yielded a score of 91, indicating excellent environmental performance with minimal penalty points associated with solvent use, waste generation, and instrument energy consumption. The AGREE assessment produced a score of 0.82, demonstrating a high degree of compliance with the twelve principles of Green Analytical Chemistry. Likewise, the GAPI pictogram revealed predominantly green zones with only minor yellow regions related to instrumental operation and solvent waste, confirming the low environmental impact of the developed procedure.

In addition to its favorable greenness profile, the method exhibited excellent analytical characteristics, including high chromatographic efficiency (plate count > 5000), acceptable peak symmetry (tailing factor 1.2), excellent precision (%RSD < 2), good linearity (r = 0.998), and satisfactory assay results (99.7%). These findings demonstrate that the adoption of green analytical strategies did not compromise analytical performance, thereby establishing the method as a reliable and sustainable alternative for routine pharmaceutical analysis [20].

SUMMARY AND CONCLUSION:

The present investigation focused on the development and validation of a simple, rapid, precise, accurate, and environmentally sustainable RP-HPLC method for the quantitative determination of Tucatinib in pharmaceutical dosage forms. Chromatographic separation was achieved on a Hypersil C18 column using an ethanol–water (65:35, v/v) mobile phase at a flow rate of 0.7 mL/min with UV detection at 260 nm. The developed method exhibited excellent chromatographic performance with a retention time of approximately 2.3 min, theoretical plate count exceeding 5000, and a tailing factor of about 1.2. Validation studies performed according to ICH guidelines demonstrated good linearity over the concentration range of 24–120 µg/mL (r = 0.998), high precision (%RSD < 2), satisfactory accuracy (98–102% recovery), and acceptable robustness and ruggedness. The assay of Tucatinib in pharmaceutical formulation was found to be 99.7%, confirming the suitability of the method for routine quality control analysis.

The environmental sustainability of the developed method was evaluated using Green Analytical Chemistry assessment tools, including Analytical Eco-Scale, AGREE, and GAPI. The method achieved an Eco-Scale score of 91 and an AGREE score of 0.82, while the GAPI assessment indicated predominantly green zones, confirming its minimal environmental impact. The replacement of conventional hazardous solvents with ethanol, coupled with reduced solvent consumption and short analysis time, significantly enhanced the greenness profile of the method. Overall, the developed RP-HPLC method successfully combines excellent analytical performance with environmental sustainability, making it a reliable, cost-effective, and eco-friendly approach for the routine determination of Tucatinib in pharmaceutical quality control laboratories.

Finally a simple, rapid, precise, and eco-friendly RP-HPLC method was successfully developed and validated for the determination of Tucatinib in pharmaceutical dosage forms. The method complied with ICH validation requirements and demonstrated excellent analytical performance, including accuracy, precision, and robustness. Green assessment studies confirmed its high environmental sustainability, with an Eco-Scale score of 91 and an AGREE score of 0.82. Therefore, the method is suitable for routine quality control analysis and represents a sustainable alternative to conventional HPLC methods.

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