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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF TUCATINIB IN BULK AND MARKETED FORMULATION BY RP HPLC METHOD

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Abstract:

An analytical, specific, accurate, precise, robust, efficient and simple RP-HPLC method has been developed and validated for the determination of Tucatinib in bulk and was applied on marketed Tucatinib products. The mobile phase used for the chromatographic runs consisted of Phosphate Buffer (0.02M) and Acetonitrile in the proportion of 48:52% with maintained at pH-2.80 by diluted OPA solution. The separation was achieved on a Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm column using isocratic mode. Drug peak were well separated and were detected by a UV detector at 248 nm. The method was linear at the concentration range 30–70 µg/ml for Tucatinib. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. Tucatinib limit of detection (LOD) and limit of quantification (LOQ) were 0.09µg/ml and 0.27µg/ml respectively.

Keywords: *Tucatinib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.*

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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. It was developed by Seattle Genetics and approved by the FDA on April 17, 2020 [1]. By inhibiting tyrosine kinase, Tucatinib exerts anti-tumor activity, reducing the size of HER-2 positive breast cancer tumors. In clinical trials, the regimen of Tucatinib and Trastuzumab showed enhanced activity both in vitro and in vivo when compared to either drug administered by itself. 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 [2]. 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 [3]. The Chemical Structure of Tucatinib is shown as follows

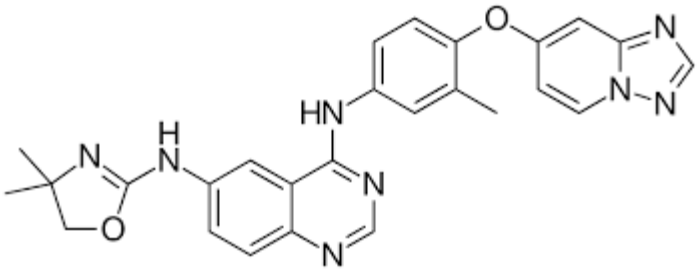


Fig-1: Chemical Structure of Tucatinib

The literature survey reveals that there are few reversed-phase high-performance liquid chromatography (RP-HPLC) methods reported for the estimation of Tucatinib in bulk form and pharmaceutical dosage form [31-32]. Thus, the present work was carried out to develop novel, precise, accurate, rapid, and cost-effective method and to validate the method for the estimation of Tucatinib in bulk and pharmaceutical dosage forms and its application for the separation of the peak of a degradation product.

MATERIALS AND METHODS

1. Instruments Used:

Table-1: List of Instrument Used

S. No.	Instruments/Equipments/Apparatus
1.	**Waters HPLC** with Empower2 Software with Isocratic with UV-Visible Detector.
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry RP C_{18}, 5µm, 250mm x 4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

2. Chemicals / Reagents Used:

Table-2: List of Chemicals Used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	96%	A.R.	Sd fine-Chem ltd; Mumbai
8.	3% Hydrogen Peroxide	96%	A.R.	Sd fine-Chem ltd; Mumbai

Method Development and Its Validation for Tucatinib by RP-HPLC:

Selection of Wavelength:

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Tucatinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Tucatinib. While scanning the Tucatinib solution we observed the maxima at 248 nm [4]. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,

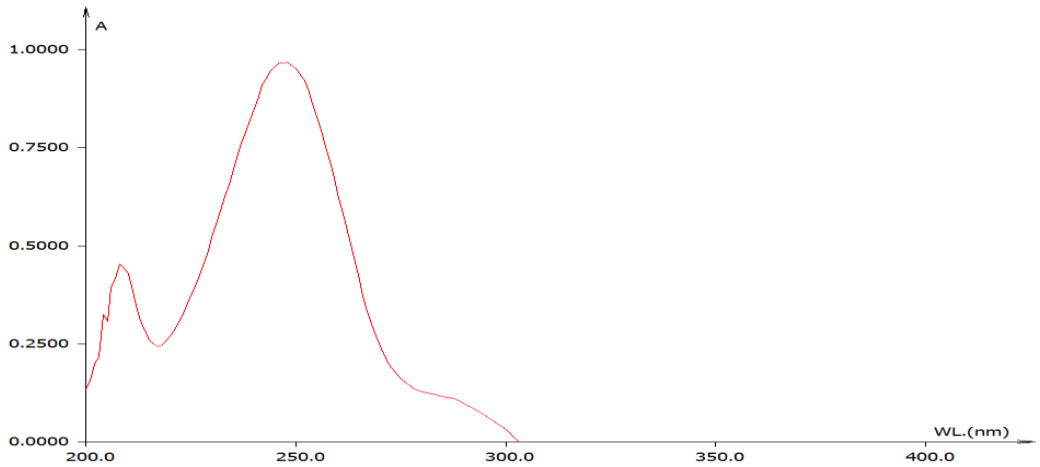


Fig-2: UV Spectrum for Tucatinib

Sample & Standard Preparation for the Analysis

25 mg of Tucatinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.3 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions:

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc. [5]

Table-3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Develosil ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Methanol : Water = 40 : 60	1.0ml/min	248nm	Very Low response	Method rejected
Zorbax ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile : Water = 60 : 40	1.0ml/min	248nm	Low response	Method rejected
Inertsil ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile: Methanol = 70 : 30	1.0ml/min	248nm	Tailing peaks	Method rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer : Acetonitrile = 30:70 (pH-4.0)	1.0ml/min	248nm	Resolution was not good	Method rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer : Methanol = 20:80 (pH-3.8)	1.0ml/min	248nm	Tailing peak	Method rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer : Acetonitrile = 48:52 (pH-2.8)	1.0ml/min	248nm	Nice peak	Method accepted

Preparation of 0.02M Potassium dihydrogen orthophosphate Solution:

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution.

Preparation of Mobile Phase:

480mL (48%) of above Phosphate buffer solution and 520mL of HPLC Grade Acetonitrile (52%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration [6-8].

Summary of Optimized Chromatographic Conditions:

The Optimum conditions obtained from experiments can be summarized as below:

Table-4: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80)
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	248 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Mode of Elution	Isocratic
Retention time	3.649 minutes

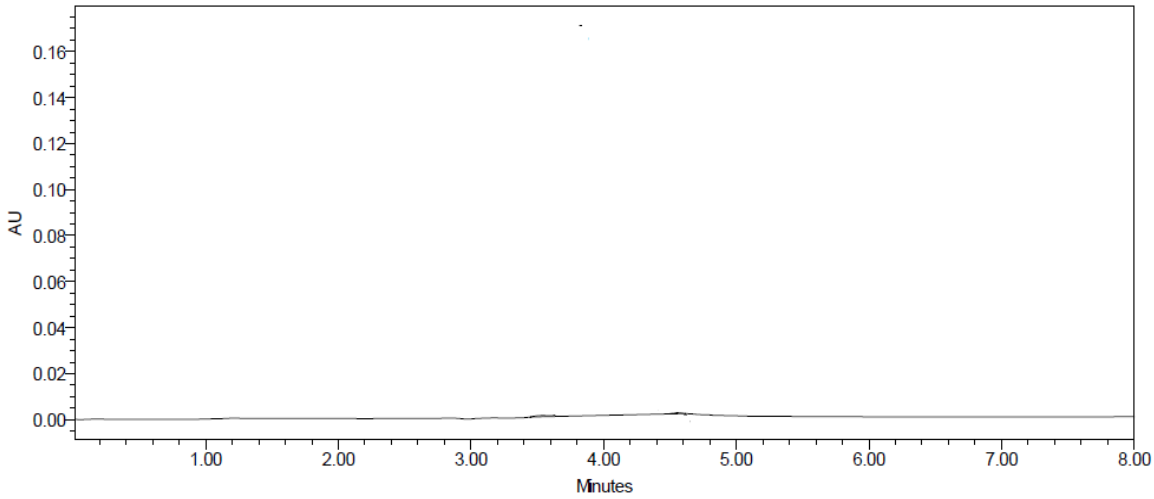


Fig-3: HPLC Spectrum of Tucatinib (Blank Solution)

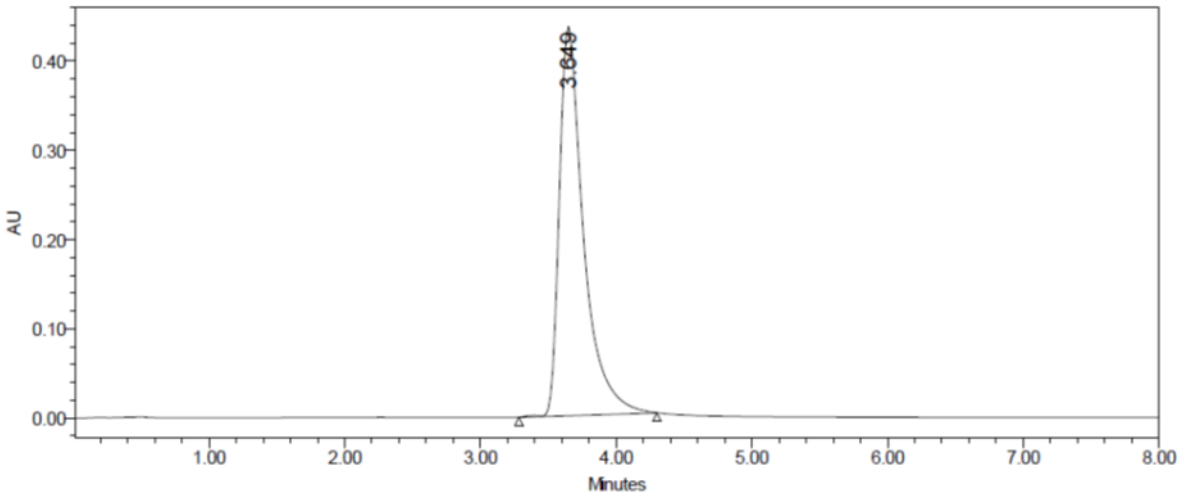


Fig-4: Chromatogram of Tucatinib in Optimized Chromatographic Condition

Method Validation:

The developed method for Tucatinib was validated for parameters such as system suitability, precision, linearity, accuracy, robustness, and solution stability as per ICH guidelines [13,14,25,30].

1. Accuracy:

Recovery Study:

To determine the accuracy of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Tucatinib were taken and extra to the pre-analyzed formulation of concentration 30µg/ml. From that proportion recovery values were calculated [9-10]. The results were shown in table-5.

Table-5: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	40	40.141	502647	100.352	Mean= 100.3947% S.D. = 0.071319 % R.S.D.= 0.071038
S_2 : 80 %	40	40.191	503214	100.477	
S_3 : 80 %	40	40.142	502656	100.355	
S_4 : 100 %	50	50.044	614215	100.088	Mean= 99.98533%

S_5 : 100 %	50	49.887	612451	99.774	S.D. = 0.183045 % R.S.D.= 0.183071
S_6 : 100 %	50	50.047	614254	100.094	
S_7 : 120 %	60	60.192	728547	100.32	Mean= 100.311% S.D. = 0.408574 % R.S.D.= 0.407308
S_8 : 120 %	60	59.939	725698	99.898	
S_9 : 120 %	60	60.429	731211	100.715	

2. Precision:

2.1. Repeatability

The exactitude of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Tucatinib (API) [11-12]. The % relative variance was calculated for Tucatinib square measure bestowed within the table-6.

Table-6: Repeatability Readings

HPLC Injection Replicates of Tucatinib	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

2.2. Intermediate Precision:

2.2.1. Intra-Assay & Inter-Assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Tucatinib revealed that the proposed method is precise [15-17].

Table-7: Results of In tra-Assay & Inter-Assay

Conc. of Tucatinib (API) (µg/ml)	Observed Conc. of Tucatinib (µg/ml) by the Proposed Method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
40	40.05	1.09	39.89	1.08
50	50.08	0.95	49.54	0.76
60	60.09	0.97	59.86	0.94

3. Linearity & Range:

The calibration curve showed good linearity in the range of 0-70µg/ml, for Tucatinib (API) with correlation coefficient (r^2) of 0.999 (Fig-5). A typical calibration curve has the regression equation of y = 11266.x + 50416 for Tucatinib [18-19].

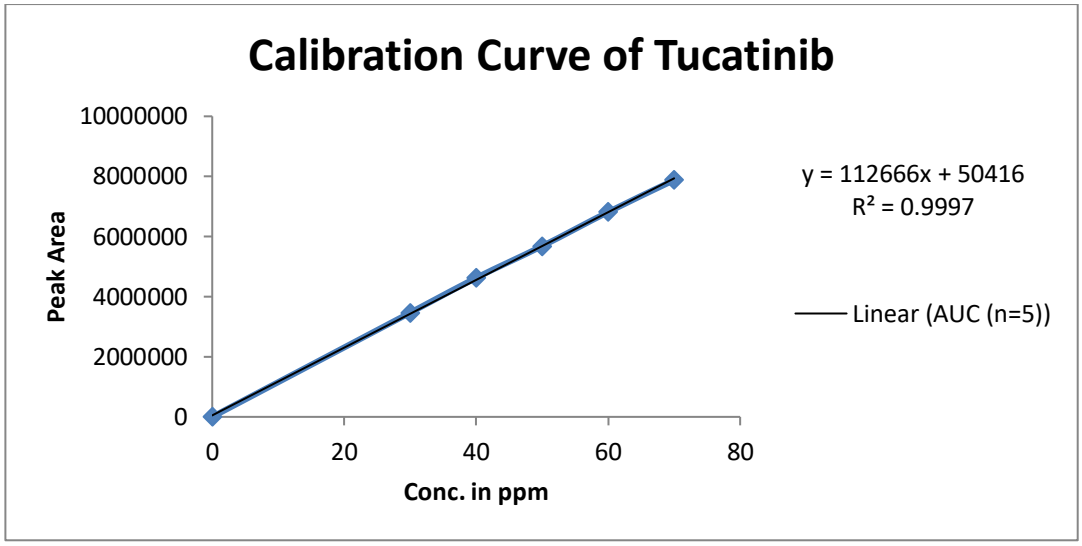


Fig-5: Calibration Curve of Tucatinib (API)

Table-8: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
30	3465974
40	4626478
50	5682284
60	6815478
70	7878721

4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & Acetonitrile content in mobile part (±2%) studied to work out the strength of the tactic also are in favour of (Table-9, nada RSD < 2%) the developed RP-HPLC technique for the analysis of Tucatinib (API) [20-22].

Table-9: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.56
Flow (0.9 ml/min)	0.87
Temperature (27^{0}C)	0.72
Temperature (23^{0}C)	0.53
Wavelength of Detection (257 nm)	0.61
Wavelength of detection (253 nm)	0.96

5. LOD & LOQ:
The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27 µg/ml respectively [23].

6. System Suitability Parameter:
System quality testing is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically. Following system quality check parameters were established [24]. The obtained information is shown in Table-10.

Table-10: Knowledge of System quality Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.54
2	Asymmetry	T ≤ 2	Tucatinib =0.98
3	Theoretical plate	N > 2000	Tucatinib =4782
4	Tailing Factor	T<2	Tucatinib =1.49

7. Estimation of Tucatinib in Pharmaceutical Dosage Form
Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to work out the typical weight. On top of weighed tablets/capsules were finally pulverized and triturated well. A amount of powder cherish twenty five mg of medicine were transferred to twenty five cc meter flask, build and resolution was sonicated for quarter-hour, there once volume was created up to twenty five cc with same solvent. Then ten cc of the on top of resolution was diluted to a hundred cc with mobile part. The answer was filtered through a membrane filter (0.45 µm) and sonicated to remove. The answer ready was injected in 5 replicates into the HPLC system and therefore the observations were recorded [26-28].
A duplicate injection of the quality resolution was conjointly injected into the HPLC system and therefore the peak areas were recorded. The info square measure shown in Table-11.

ASSAY:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:
AT = Peak space of drug obtained with check preparation
AS = Peak space of drug obtained with normal preparation
WS = Weight of operating normal taken in mg
WT = Weight of sample taken in mg
DS = Dilution of normal resolution
DT = Dilution of sample resolution
P = proportion purity of operating normal

Table-11: Recovery Data for Estimation Tucatinib [29-30]

Brand Name of Tucatinib	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the Proposed Method (n=6)	Assay % (± SD)
Tucaone 150mg Tablet (MSN Laboratories)	150mg	149.598 (± 0.269)	99.348 (± 0.695)

Observation: The amount of drugs in Tucaone 150mg Tablet was found to be 149.598 (± 0.269) mg/tab for Tucatinib & % assay was 99.348%.

SUMMARY:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 246nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry C18 ODS (4.6mm×250mm) 5µm particle size because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile: Phosphate buffer (0.01M, & pH-3.2) (30:70 v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 10min because analyze gave peak around 5.453min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of the Tucatinib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

BIBLIOGRAPHY:

1. https://go.drugbank.com/drugs/DB11652
2. https://pubchem.ncbi.nlm.nih.gov/compound/Tucatinib
3. https://en.wikipedia.org/wiki/Tucatinib
4. R. Snyder, J. Kirkland, L. Glajch, Practical HPLC Method Development, john Wiley and sons International publication, II Edn., 2011.
5. S. Ashutoshkar, Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers, 452-474, 2005.
6. H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors', New Delhi. 1-9, 157-167.
7. H.H. Williard, L.L. Merit, F.A. Dean, F.A. Settle, Instrumental Methods Of Analysis, 6th Edn, C.B.S. Publishers and Distributors, New Delhi.: 430-440, 495-504,529-545.
8. B.K. Sharma, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut: 286-300.
9. Instant notes on analytical chemistry by D. Kealey and P.J. Haines, © BIOS Scientific Publishers Limited, UK, 6-7, 2002.
10. Gurdeep R. Chatwal, Sham K. Anand, Instrumental methods of Chemical Analysis,5th edition, Himalaya Publishing House(Mumbai), P-2.566, 2005,.
11. M. E. Swartz, Journal of liquid chromatography, 28(7/8), 1253-1263(2005).
12. Journal of Chromatography .B, Analytical Technologies in the Biomedical and life Sciences. 2008 March 1; 863(2): 258-265. Published on Jan 18 2008.
13. International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1). November 2005.
14. International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A. 1994.
15. J. M. Green, a practical guide to analytical method validation, anal. Chem. News & features, pp. 305a–309a, 1 may 1996.
16. P. A. Winslow and r. F. Meyer, defining a master plan for the validation of analytical methods, j. Validation technology, pp. 361–367, 1997.
17. Aoac peer-verified methods program, manual on policies and procedures, Arlington, Va., USA (1998).
18. R. Patil: J of Chromatographia, 67, 575, (2008).
19. Baht and Leena: J of Liq. Chrom., 30, 309, (2007).
20. H.H. Williard, L.L. Merit, F.A. Dean and F.A. Settle, Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi, 2002.
21. GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).
22. Food and Drug Administration (FDA), "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices), Vol: 65 (169), 52776–52777, 2000.
23. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
24. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
25. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
26. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
27. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al.

International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
28. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC ChromAcademy.
29. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application-A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
30. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
31. S. K. Reehana1*, K. Sujana1, LC-MS/MS Characterization of Forced Degradation Products of Tucatinib, a Novel Tyrosine Kinase Inhibitor: Development and Validation of Rp-Hplc Method, International Journal of Applied Pharmaceutics, Vol 14, and Issue 1, 2022, 58-66.
32. Bandaru Venkata Ramarao, Anand Solomon Kamalakaran*, Development and validation of an LC-MS/MS method for pharmacokinetic assessment of Tucatinib in rat plasma, Journal of Applied Pharmaceutical Science, Vol. 15(04), pp 225-233, April, 2025.