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HPLC Method Development and Validation for Quantitative Estimation of Dacomitinib in Pharmaceuticals Dosage Form

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ABSTRACT

The development of pharmaceutical products brought a revolution in human heath, but controlling the concentration of drugs in these products is essential to patient's safety. In this study, a simple, sensitive and specific HPLC method is developed and validated to quantitatively estimate dacomitinib in the pharmaceuticals dosage form. Chromatographic separation was performed on an Kromasil C_{18} Column (250 x 4.6 mm; 5µm) via isocratic elution with mobile phase consisting of 0.2% triethylamine solution (pH = 3.0 adjusted with 85% orthophosphoric acid): acetonitrile (70:30) with a flow rate of 1mL/min. The detection was achieved with UV/Vis detector with a detection wavelength of 260 nm. The method was validated in terms of linearity, sensitivity, precision, accuracy and limit of quantification tests. Dacomitinib can be successfully separated with good linearity (the regression equation is A =7566.3C + 1610.0; R^2 = 1.0) and perfect recovery with the results of accuracy were in the range 98-102%.

INTRODUCTION

Cancer is a leading public health problem worldwide and Lung cancer (both small cell and non-small cell) is the second most common cancer in both men and women. In men, prostate cancer is more common, while in women breast cancer is more common [1]. Non-small cell lung cancer (NSCLC) accounts for nearly 85% of all new cases diagnosed. Survival has not significantly improved in the past decades, most patients are diagnosed at a late-stage disease when surgery is no longer feasible and consequently have poor prognosis [2]. The development of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) has transformed the management of advanced non–small-cell lung cancer (NSCLC) bearing an activating EGFR mutation, offering improved survival and tolerability when compared with the use of up-front cytotoxic chemotherapy [3]. First-line TKIs controlling EGFR (e.g., erlotinib and gefitinib) have good initial responses against these mutations [4]. Unfortunately, Acquired resistance after treatment with first-generation EGFR TKIs led several groups to develop second-generation EGFR TKIs (e.g., dacomitinib (DMB) and Afatinib) [5].

Dacomitinib (CAS 1110813-31-4) is an oral small molecule second-generation pan-HER TKI that irreversibly and selectively binds to the ATP binding pockets of EGFR, ERBB2 and ERBB4 at low nanomolar affinities [6-7]. In a recent phase III clinical trial dacomitinib was found to produce a remarkable increase in progression-free survival (PFS) in the first line treatment of patients with advanced EGFR-mutated non-small cell lung cancer as compared with gefitinib [8]. The longer duration of response recorded with dacomitinib compared to gefitinib in patients who responded to treatment might be due to the irreversible binding of dacomitinib to its targets in contrast to the reversible binding of gefitinib [9]. Patients received a range of dacomitinib from 0.5 to 60 mg/day. Dacomitinib had a long half-life of 59–85 h and an apparent volume of distribution of approximately 2,600 L over the 30-60 mg dosage range. Given the long half-life, accumulation was expected and maximal accumulation was observed during cycle 1. No effects of food or locally acting antacids on the pharmacokinetics of dacomitinib were seen in this study [10].

On September 27, 2018, the Food and Drug Administration (FDA) approved dacomitinib (DMB), a potent and irreversible second-generation EGFR-targeted TKI agent in the form of VIZIMPRO tablets. The irreversible pan-human epidermal growth factor receptor (HER) family inhibitor includes the first-line treatment of patients with metastatic NSCLC harboring EGFR Del19 or exon 21 L858R mutations [2,4]. The chemical name of Dacomitinib is (2E)-N-

{4-[(3-Chloro-4-fluorophenyl)amino]-7-methoxy-6-quinazolinyl}-4-(1-piperidinyl)-2-butenamide with its empirical formula is $C_{24}H_{25}ClFN_5O_2$ and molecular weight of 469.94.

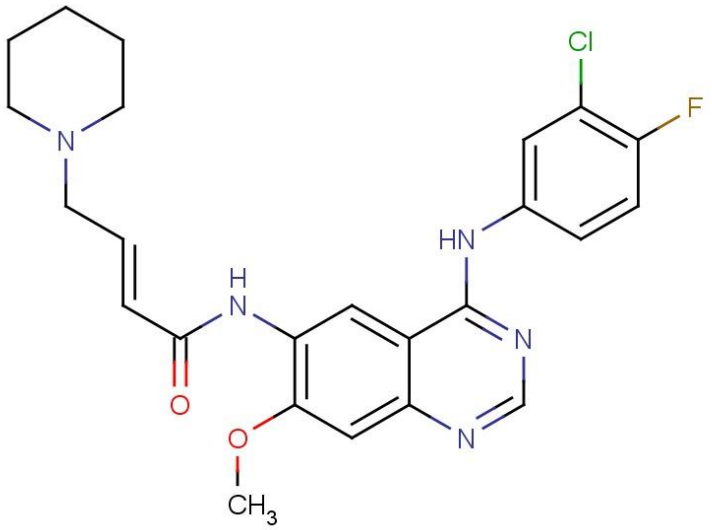

Figure No.1: Structure of Dacomitinib

To the best of our knowledge, a single validated LC-MS/MS assay was lately published reporting the assay for quantification of Dacomitinib and application to investigating its metabolic stability [4]. The purpose of the present study was to develop a validated HPLC Method for quantitative assay estimation of dacomitinib in a pharmaceuticals dosage form. The HPLC method described here is simple, sensitive, and reproducible for Dacomitinib determination in Formulations with low background interference.

MATERIALS AND METHODS

Chemicals and reagents

Dacomitinib active pharmaceutical ingredient (API), was procured from Central Drugs Testing Laboratory-Mumbai. Dacomitinib Tablets were purchased from local market containing 45 mg of Dacomitinib per tablet. Triethylamine (AR grade) from Rankem, Acetonitrile (HPLC grade) and Orthophosphoric acid from Molychem were used. Ultra-purified HPLC grade distilled water obtained from the Milli-Q® system (Millipore, Milford, MA, USA) water purification unit was used to prepare all the required solutions.

Determination of wavelength

The standard solution 100 ppm of Dacomitinib was scanned in the range of 400 to 210 nm against acetonitrile as a blank. Dacomitinib showed maximum absorbance at 260 nm as

shown in **Figure 2.** So, the suitable wavelength selected for the HPLC analysis of Dacomitinib was 260 nm.

Instrumentation and chromatographic conditions

The HPLC analysis of Dacomitinib was carried out using Thermo dionex ultimate 3000 system equipped with an auto-injector and four variable UV/VIS dual-wavelength detectors. The column used for the analysis was a Kromasil 100_5_C18 with 5 µm particle size, 4.6 mm internal diameter and 250 mm length, which was in an oven at a temperature of 40ºC. The data were recorded using Chromeleon 7.2 software. Chromatographic analysis was conducted in Isocratic mode. Preferentially, the UV detector was set at 260 nm for the detection of Dacomitinib and the injection volume was 20 µL for standard and samples. Before analysis, every standard and sample were filtered through 0.22 µm filters.

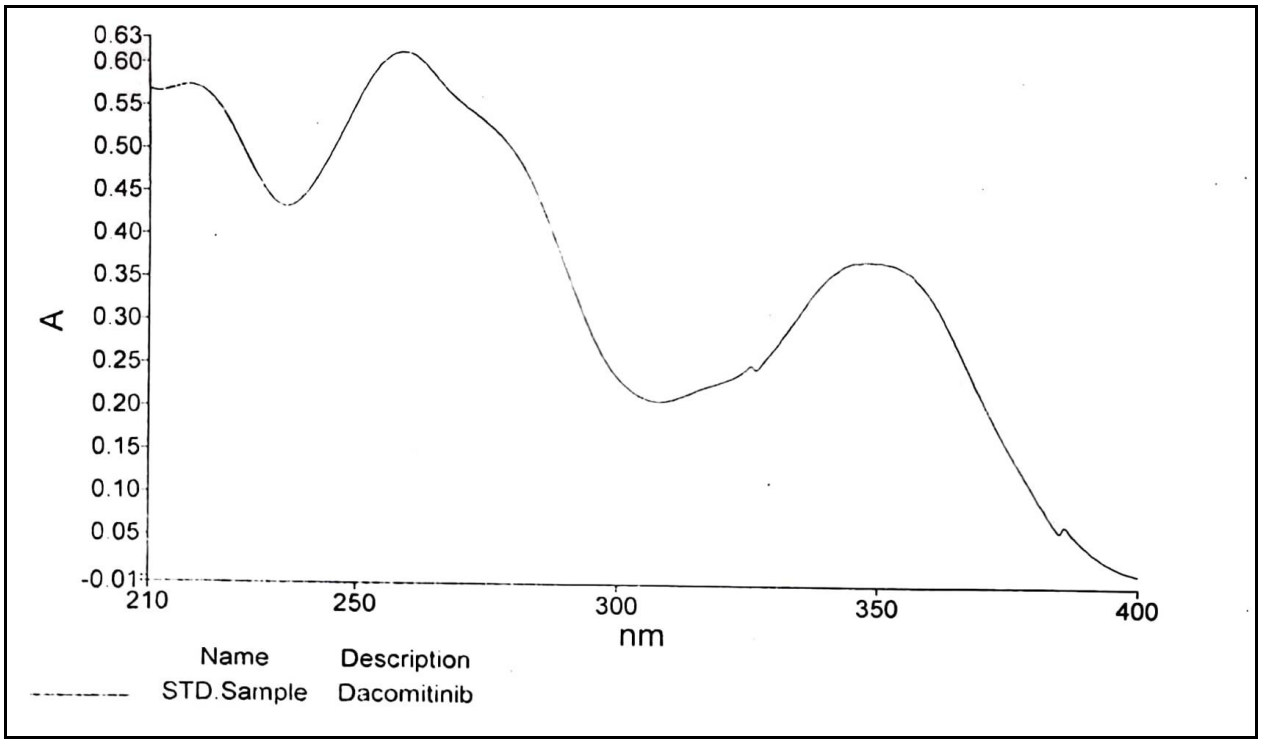

Figure No.2: Wavelength Scan of Dacomitinib.

Mobile Phase Preparation

The mobile phase consisted of a mixture of a buffer and acetonitrile with a proportion of (70:30). The buffer was prepared as 0.2% v/v solution of Triethylamine with pH adjusted to 3.2 ± 0.1 with phosphoric acid. All solutions were filtered through a 0.22 membrane filter and sonicated to degas.

Preparation of Standard Solution

The standard solution was prepared by accurately weighing and dissolving 10 mg of Dacomitinib in a 100 ml volumetric flask with 30 ml of Acetonitrile and make up to the mark with the buffer solution. The solution was then diluted with the mobile phase to obtain a Dacomitinib concentration of 30 µg/ml.

Preparation of Sample Solution

An amount of Dacomitinib (equivalent to about 30 mg) was diluted to 100 ml with 30 ml of Acetonitrile and make up to the mark with the buffer solution, mixed and filtered. The solution was then diluted with the mobile phase to obtain final concentration of 30 µg/ml.

Method Development

Molecular structure and solubility data show that Dacomatinib is a weekly acidic molecule with very low water solubility. Initial trials on HPLC were carried out using synchronize C18 (4.6mm X 150mm,5 µ) Merck Make column, with a mobile phase consisting of buffer (0.2% v/v triethylamine in water at different PH ranging from 3.0 to 4.5) with varying proportions of acetonitrile. In the chromatogram obtained by following the above conditions, early retention of peak with asymmetry was observed. Later trials were conducted on the Merck Purospperstar C18 (4.6mm X 250mm, 5 µ). In chromatogram recorded Capmatinib peak was obtained was having unacceptable SST parameters. Further trials were conducted using Kromasil- C18 (4.6 mm x 150 mm, 5µ) with a mobile phase consisting of buffer (0.2% triethylamine in water at different PH ranging from 3.0 to 4.5) with varying proportions of acetonitrile. Chromatograms with accepted peak shape and SST Parameters were obtained on this column. Hence finally Kromasil C18 (4.6 mm x 150 mm, 5µ) column and mobile Phase consisting of buffer (0.2% triethylamine in water, adjusted to pH 3.0) and acetonitrile was selected for the study.

Method Validation

The method was validated for system suitability testing, specificity, linearity, precision, accuracy and recovery, LOD, LOQ, and robustness parameters according to ICH Q2 (R1) guidelines.

System Suitability Testing

System suitability parameters were evaluated and analyzed to check the system performance by injecting a working standard solution (five replicate) of a concentration of 30 µg/ml and blank preparation (single injection) into the HPLC. The chromatograms were recorded to evaluate SST parameters like % RSD of Retention time, Tailing factor, Theoretical plates. Data of system suitability studies are summarized in **Table 1**.

Table No.1: System suitability and system precision study of Dacomitinib

Injection no	Area	Retention Time	Theoretical Plates	Tailing Factor
1	224731	5.40	9454	1.40
2	224858	5.39	9583	1.166
3	224728	5.40	9382	1.153
4	224903	5.39	9336	1.135
5	225136	5.39	9409	1.159
AVERAGE	224871.2	5.394	9432.8	1.151
SD	166.93	0.005477	94.24	0.01
% RSD	0.07	0.10	1.00	1.12
LIMIT	NMT 2.0%	NMT 1.0%	NMT 2.0%	NMT 2.0%

Specificity

For specificity solutions of blank, a standard of 30 µg/ml and a sample of 30 µg/ml were injected and their chromatograms were recorded as represented in **Figures 3, 4, 5** respectively. This indicates that the blank (diluent solution) used in sample and standard preparation do not interfere in the estimation of Dacomitinib in its tablet formulation.

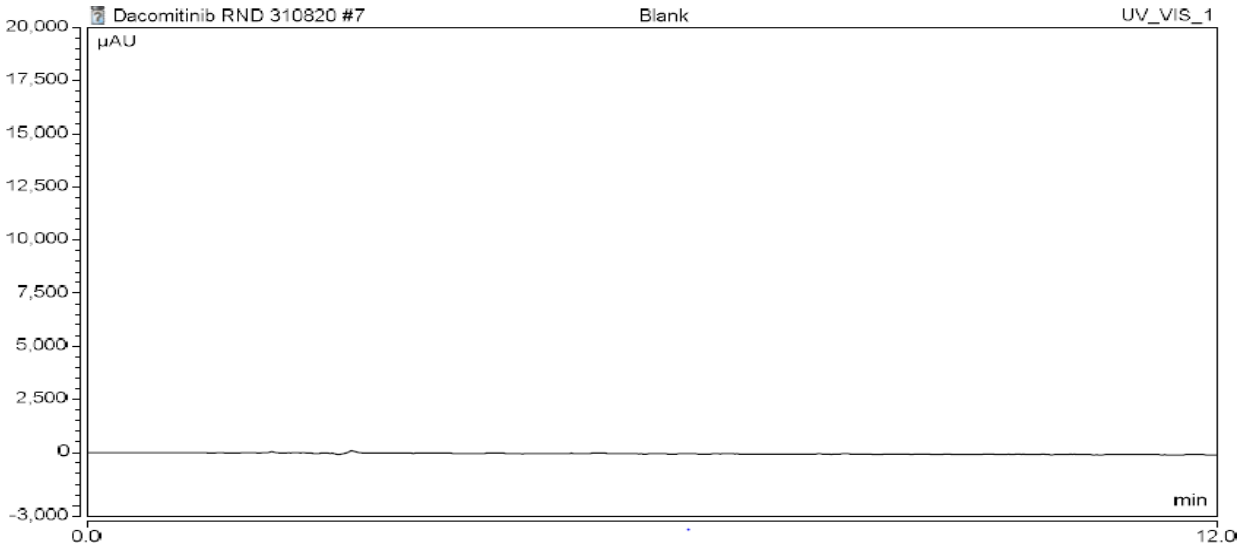

Figure No. 3: Chromatogram of Blank solution

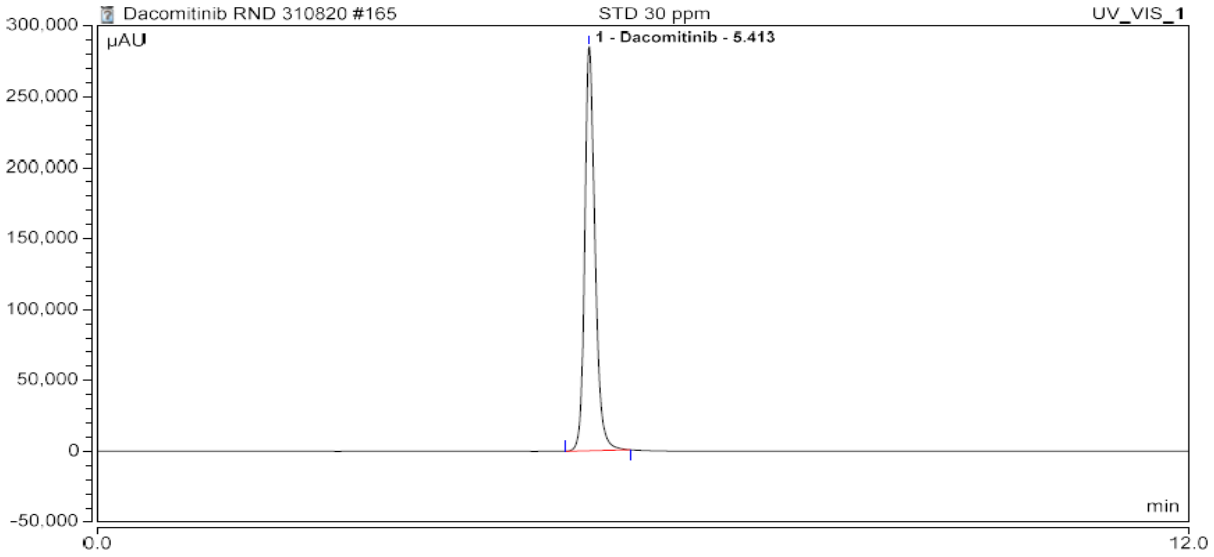

Figure No. 4: Chromatogram of Standard solution

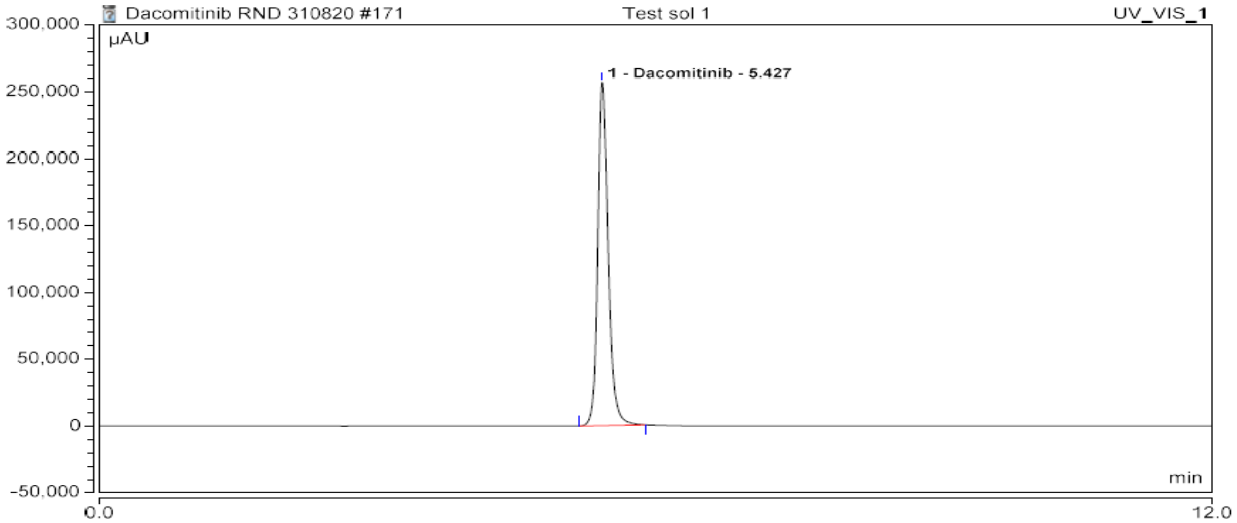

Figure No. 5: Chromatogram of Sample solution

Linearity

The standard curve was obtained within the concentration range of 5-75 µg/ml for Dacomitinib. The linearity of this method was evaluated by linear regression analysis. The linearity graph was plotted by taking the concentration of the drug on the X-axis and the corresponding peak area on the Y-axis as shown in **Figure 6**. The linearity data is summarized in **Table 2**.

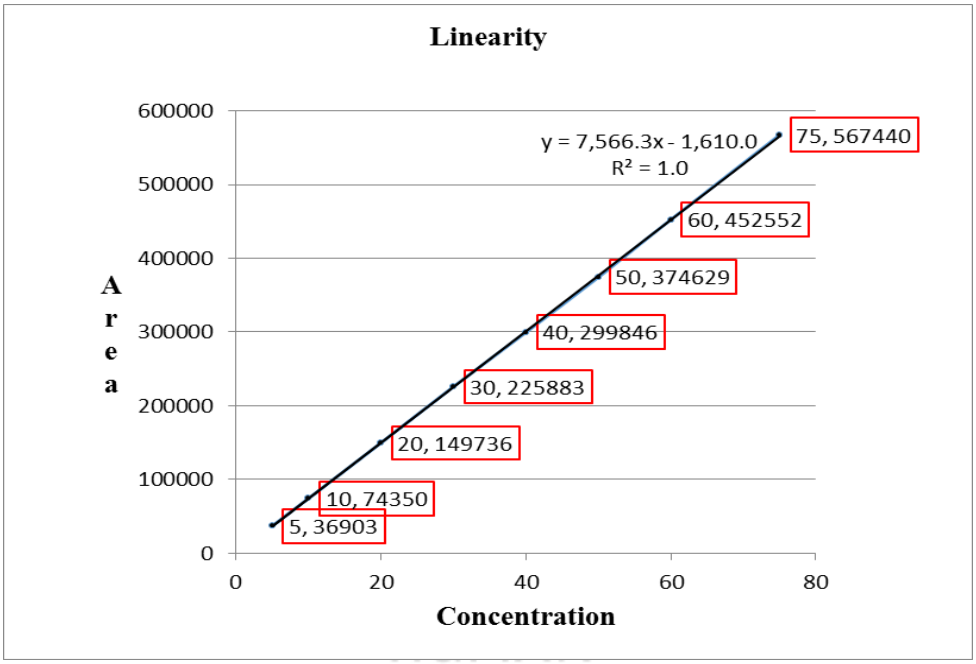

Figure No. 6: Linearity graph of Dacomitinib

Table No. 2: Linearity data of Dacomitinib	
Concentration (µg/ml)	**Area**
5	36903
10	74350
20	149736
30	225883
40	299846
50	374629
60	452552
75	567440

Precision

"The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample".

System Precision: This was performed by injecting six replicate injections of a standard solution (30µg/ml). The average, SD, % RSD of an area in six replicate injections was calculated and reported. The results are summarized in **Table 1**.

Method Precision (Assay Repeatability): This was performed by injecting five replicate injections of standard solution (30 µg/ml) and six sample preparation of Dacomitinib (30 µg/ml) in triplicates into the HPLC system. Its % Assay, average, SD, %RSD were calculated and reported. The mean assay percentage results are summarized in **Table 3.**

Table No. 3: Method Precision (Assay Repeatability) data of Dacomitinib

Sample No.	% Assay
1	99.71
2	100.48
3	99.50
4	99.35
5	100.38
6	100.29
AVERAGE	99.95
SD	0.448
%RSD	0.45
Limit	NMT 2%

Intermediate Precision: This was performed on two different days, by two different analysts, and different HPLC instruments. Five replicates of standard solution (30 µg/ml) and three sample preparation (30 µg/ml) in triplicates were injected into the HPLC system. Its % Assay, average, SD, %RSD were calculated and reported. Results are summarized in **Table 4.**

Table No. 4: Intermediate precision data of Dacomitinib				
Sample No.	**% Assay Day-1 HPLC-1**	**% Assay Day-2 Analyst -1**	**%Assay HPLC-1 Analyst-2**	**% Assay Analyst -1 HPLC-2**
1	99.71	100.1	99.91	100.48
2	100.48	100.3	100.19	100.06
2	99.50	100.1	99.60	100.16
AVERAGE	99.90	100.16	99.90	100.23
SD	0.52	0.14	0.238	0.22
%RSD	0.520	0.136	0.24	0.222
LIMIT	NMT 2%	NMT 2%	NMT 2%	NMT 2%

Accuracy and Recovery:

Accuracy is a measure of how close is the experimental value to the true value. Accuracy was determined by the method of standard addition method, by calculating of % mean recovery of the sample at Four different levels 100, 110, 120, 130%. At each level, three determinations were performed, the amount recovered, % recovery and % RSD were taken into consideration. Accuracy results at various levels of concentration are summarized in **Table 5.**

Table No. 5: Accuracy data of Dacomitinib						
% Level	**%Amount Recovered**	**% Recovery**	**AVERAGE**	**SD**	**%RSD**	**%Mean Recovery**
100	45.21	100.5	100.3	0.074	0.074	100.10
	45.15	100.3				
	45.19	100.4				
110	49.55	110.1	100.0	0.072	0.072	
	49.49	110.0				
	49.48	110.0				
120	54.01	120.0	99.7	0.183	0.184	
	53.81	119.6				
	53.91	119.8				
130	58.62	130.3	100.2	0.156	0.156	
	58.63	130.3				
	58.47	129.9				

LOD and LOQ:

Limit of detection (LOD) and limit of quantification (LOQ) of Dacomitinib were determined from the calibration curve method using the following formulas:

$$LOD = 3.3 \times \propto/s,\ LOQ = 10 \times \propto/s$$

Where $\propto$ is the Standard deviation of the response of the regression line and s is the slope obtained from the calibration curve. After calculating, solutions of desired concentration for LOD and LOQ were prepared and injected. The chromatograms obtained were recorded as represented in **Figures 7, 8.**

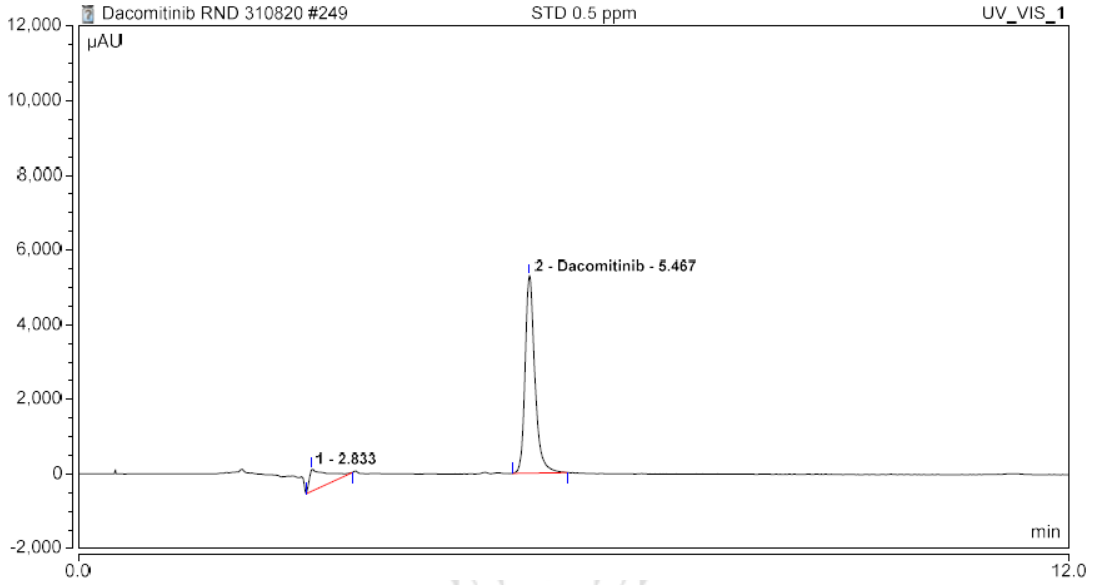

Figure No. 7: Chromatogram of LOD

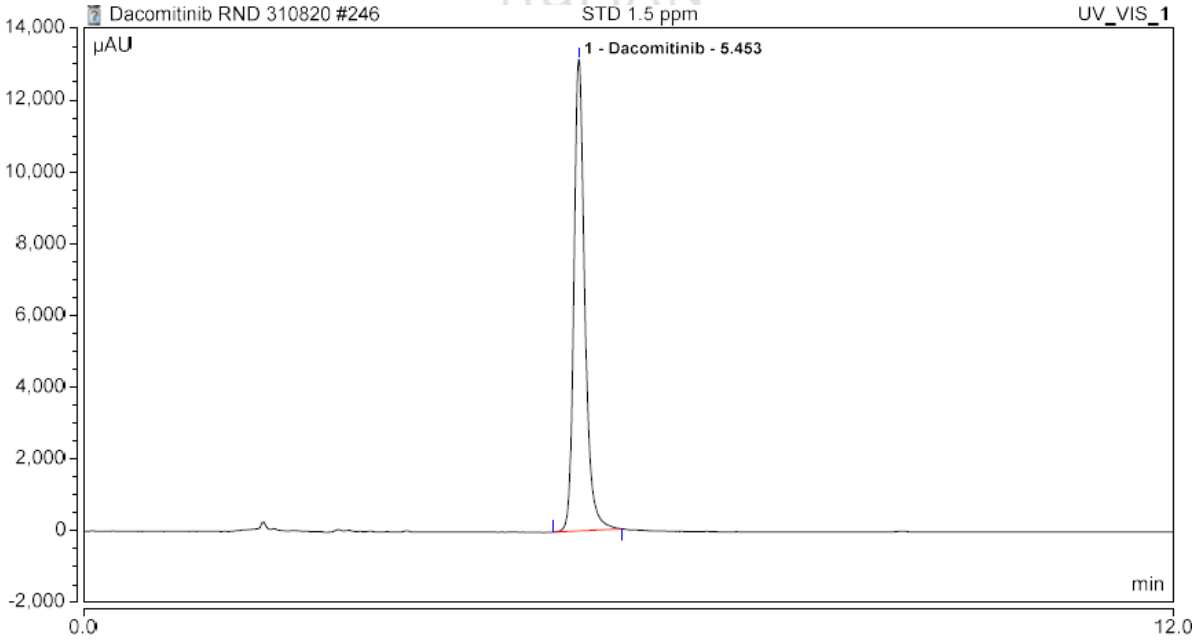

Figure No. 8: Chromatogram of LOQ

Robustness:

This was performed by a change in flow rate (± 0.2 ml/min), change the column in temperature (±5 °C), change in wavelength (±2 nm), Change in PH (± 0.2 nm), and change in the column. Three sample preparations of 30 µg/ml were prepared and injected in triplicate along with five replicate injections of a standard solution of 30 µg/ml under different chromatographic conditions. Its % assay, average, SD, %RSD were calculated and reported, results are summarized in **Table 6**.

Assay: The optimized method was applied on tablets having a label claim of Dacomitinib 45 mg. The assay was performed on the above solution five replicate injections of standard preparation 30 µg/ml and six sample preparation 30 µg/ml in triplicate were injected into the HPLC system. Its % assay, average, SD, %RSD were calculated and reported, results are summarized in **Table 7.**

Table No. 6: Robustness data of Dacomitinib

Parameter	Change in parameter (±)	% Assay Estimation	AVERAGE	SD	% RSD	LIMIT
Flow rate (±0.2 ml/min)	0.8	99.57	99.58	0.05	0.05	NMT 2%
	1	99.63				
	1.2	99.54				
Column temperature (±5°C)	35	100.90	100.3	0.90	0.89	
	40	99.27				
	45	100.73				
Wavelength (±2 nm)	258	99.55	99.80	0.68	0.68	
	260	99.27				
	262	100.57				
PH (±0.2 ml/min)	2.8	100.65	100.57	0.31	0.31	
	3	100.23				
	3.2	100.84				
Different Column	-	99.88	99.96	0.11	0.114	
	-	99.29				
	-	100.09				

Table No. 7: Assay results of Dacomitinib					
Sample No.	**Weight of standard (mg)**	**Sample weight (equivalent to 150 mg of Capmatinib)**	**Mean Area of the standard at 255 nm**	**Area of a sample at 255 nm**	**% Assay**
1	10.06	183.1	223729	199099.3	91.71
2		185.5		203275	100.48
3		181.8		197262.3	99.50
4		184.9		200332.3	99.35
5		186.5		204152.3	100.38
6		184.0		201236.3	100.2
Mean					99.95
± SD					0.448
% RSD					0.45

RESULTS AND DISCUSSION

An RP-HPLC method for quantitative estimation of Dacomitinib was developed and validated as per ICH Q2 (R1) guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. As there is no interference of blank at the retention time of Dacomitinib hence method was specific. The retention time of Dacomitinib was found around 5.3 minutes. Linearity was observed over a concentration range of 5-75 µg/ml. The correlation coefficient was found to be 0.99998.

The relative standard deviation for system suitability testing and system precision studies was found within a limit that is not more than 2%. Theoretical plates were found to be greater than 6000, also the tailing factor was reported to be less than 2. In Method precision (assay repeatability) studies of Capmatinib average assay percentage was found to be 99.95% which was within the limit i.e., between 98% to 102%. The relative standard deviation for all intermediate precision parameters was found to be within limit.

The accuracy studies were shown as % recovery at 100% to 130% level for Capmatinib. The mean percent recovery was found to 100.10% which was within limit. Hence the method was found to be accurate. The present method can detect and quantify the analyte at a lower

concentration. Limit of detection and limit of quantification values were estimated as following LOD = 0.5 µg/ml, LOQ = 1 µg/ml where standard deviation ($\propto$= 1229.47) and slope (s = 7566.28) values were obtained by calibration curve method. By analyzing robustness, resulting values were found to be within a limit that is less than 2%, thus the developed method was proved to be robust. The results obtained from assay show that the percentage recoveries were high and SD values are very low, which confirms that the method is suitable for routine analysis of Dacomitinib in its pharmaceutical preparation.

CONCLUSION

The RP-HPLC method development was found to be simple, precise, rapid, accurate for the quantification of Capmatinib in a liquid dosage form. The method was reliable in terms of system suitability, linearity, precision, accuracy and recovery, robustness, and assay. All the verification parameters were within the range according to ICH Q2A (R1) guidelines. Hence, we can conclude that the proposed RP-HPLC method can be used for routine analysis in the Pharmaceutical industry.

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