METHOD DEVELOPMENT FOR DETERMINATION OF %DRUG RELEASE IN DISSOLUTION FOR AFATINIB TABLETS 20 mg AND 50 mg BY RP-HPLC WITH PDA DETECTOR

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ABSTRACT:

Background: This paper summarizes the method development for determination of %drug release of dissolution for afatinib tablets 20 mg and 50 mg by HPLC.

Methods: Afatinib tablets were tested using a Zorbax SB C18 (150 mm X 4.6 mm), 5 µm stationary phase. At a flow rate of 1ml/min, the mobile phase was composed of acetonitrile and buffer solution in a 60:40 (percent v/v) ratio. The elution was measured with a PDA detector at 260nm detection wavelength. International Council for Harmonization (ICH) guidelines were used to validate the proposed approach.

Results: The chromatographic peaks of afatinib in this investigation showed good resolution with a retention duration of 3.9 min. Afatinib had excellent linearity, with a correlation value of 0.9997. Other validation factors, such as precision, specificity, accuracy, and robustness, indicated good dependability in Afatinib quantification.

Conclusion: According to methodology, the HPLC method for dissolving afatinib tablets has been approved. The method is determined to be useful for intended purposes and is particular, exact, robust, linear, and accurate in the 20 to 150 percent level.

Key words: Afatinib Tyrosine kinase inhibitor, Dissolution, Validation, RP-HPLC**,** PDA Detector.

INTRODUCTION:

The active ingredient in GILOTRIF tablets is afatinib, a 4-anilinoquinazoline tyrosine kinase inhibitor. Afatinib is provided as the dimelate salt; its chemical name is 2-butenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]] 7-[[(3S)-tetrahydro-3-furanyl]oxy] -6-quinazolinyl] -4-(dimethyl amino)-,(2E)- (2Z) 2 butynedioate (1:2). The empirical formula for afatinib dimaleate is C32H33ClFN5O11 and the molecular weight is 718.1 g/mol. [1-8]. Afatinib is also being studied for head-and-neck and breast malignancies, among others. [9-12]

Afatinib is an efficient inhibitor of the EGFR of the ErbB family tyrosine kinase. There are four EGFRs in humans that have a structural similarity: HER-1 (ErbB1), HER-2 (ErbB2), HER-3 (ErbB3), and HER-4

(ErbB-4) (ErbB-4). Targeting the ErbB family of growth factor receptors is essential for the development of therapeutics for epithelial malignancies due to the fact that aberrant signaling through these receptor kinases is responsible for a wide range of epithelial cancers. [13-19] Afatinib is an irreversible EGFR tyrosine kinase inhibitor that forms a covalent adduct with the active site sulfhydryl group via a Michael addition process (vide infra).

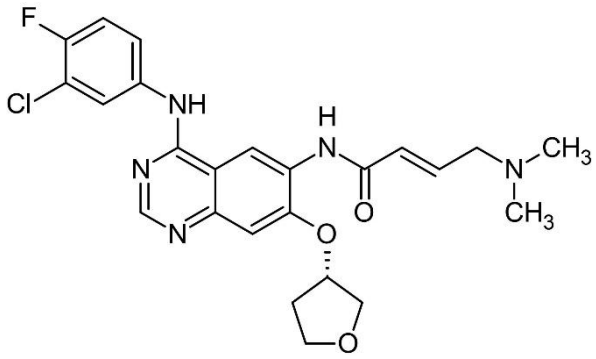


Figure 1: Structure of Afatinib

MATERIALS AND METHOD:

Chemicals and Reagents: Acetonitrile, Glacial Acetic acid, triethyl amine, ammonium acetate with water.

Instrumentation:

A high-performance liquid chromatography system with the ability to elute samples using gradient and isocratic methods, as well as an auto sampler and spectrophotometric UV detector (Waters 2998 HPLC). system for handling data (Empower 3).

Preparation of Solutions:

Preparation of Buffer solution:

Accurately weigh 3.85g of ammonium acetate and transfer it, together with 1.0 ml of triethyl amine that has been sonicated, into 1000 mL of Milli-Q water. Using glacial acetic acid, the solution's pH is adjusted to 4.5 ± 0.05. Use a 0.45 micron membrane filter to filter the mixture.

Preparation of Mobile Phase:

Prepare a homogenous mixture of acetonitrile and buffer solution in a 60:40 (percent v/v) ratio, then sonicate it for five minutes to degas it. .

Chromatographic conditions:

Column	: Zorbax SB C18 (150 mm X 4.6 mm), 5µm
Flow Rate	: 1.0 mL/ min
Injection Volume	: 10 µL

Column oven Temperature : 35°C

Sampler cooler Temperature : 5°C

Wavelength : 260 nm

Runtime : 10 Minutes

Elution : Isocratic

Preparation of Dissolution Medium :

Preparation 0.2 M Di sodium hydrogen phosphate buffer:

Disodium hydrogen phosphate anhydrous should be weighed and dissolved in 1000 mL of water.

Preparation of 0.1 M citric acid buffer:

19.21 g of citric acid anhydrous should weigh out and dissolve in 1000 mL of water.

Preparation of pH 4.0 McIlvaine buffer :

Prepare the necessary volume of a 615:385 percent v/v mixture of 0.1 M citric acid buffer and 0.2 M di sodium hydrogen phosphate buffer, and then adjust the pH to 4.0 ± 0.05 with either one of the solutions.

Preparation of Diluent :

Use dissolution medium as diluent .

Preparation of Standard solution-1:

Transfer 41.0 mg of afatinib dimaleate standard, or 28.0 mg of afatinib, accurately weighed and measured into a 100 mL volumetric flask. To completely dissolve the material, add 70 mL of solvent. Pipette 2 mL of the aforementioned standard stock solution into a 50 mL volumetric flask, diluent it to volume, and then combine it. Use a 0.45 micron filter to filter the fluid .

Preparation of Standard solution-2:

Follow procedure as per the Preparation of standard solution-1.

Dissolution Conditions :

Dissolution medium : pH 4.0 McIlavine buffer

Dissolution volume : 900 mL

Temperature : 37.0 ± 0.5°C

Apparatus : USP type I (Basket)

RPM : 100

Single point : 15 minutes

Blank preparation: Use dissolution medium as blank

Test preparation for 20 mg:

Each dissolution vessel should receive 900 mL of dissolution media. Permit the medium to reach a temperature of 37.0 ± 0.5 °C. Accurately weigh and record the individual weights of six tablets . Transfer one tablet to each dissolution vessel, cover each vessel, and operate the equipment for the designated duration. Filter the solution through a 0.45 m PVDF filter after removing 10 ml of sample from each vessel at the appropriate intervals. Add 5 ml of the previously stated filtered solution to a 10 ml volumetric flask, diluent it to volume, and thoroughly mix it.

Test preparation for 50 mg:

Each dissolution vessel should receive 900 mL of dissolution media. Permit the medium to reach a temperature of 37.0 ± 0.5 °C. Accurately weigh and record the individual weights of six tablets. Transfer one tablet to each dissolution vessel, cover each vessel, and operate the equipment for the designated duration. Filter the solution through a 0.45 m PVDF filter after removing 10 ml of sample from each vessel at the appropriate intervals. Add 2 ml of the previously stated filtered solution to a 10 ml volumetric flask, diluent it to volume, and thoroughly mix it.

Procedure: Equilibrate the HPLC system and inject into it .The retention time of Afatinib peak at about 3.9 minutes.

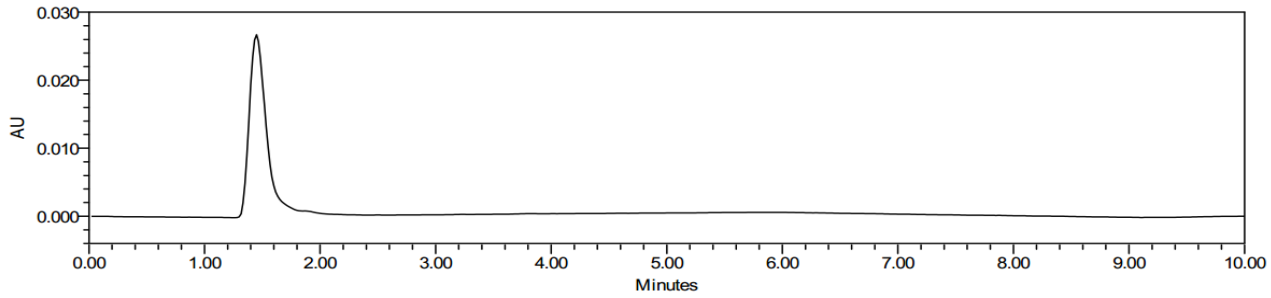


Figure 2: Typical chromatogram of Blank

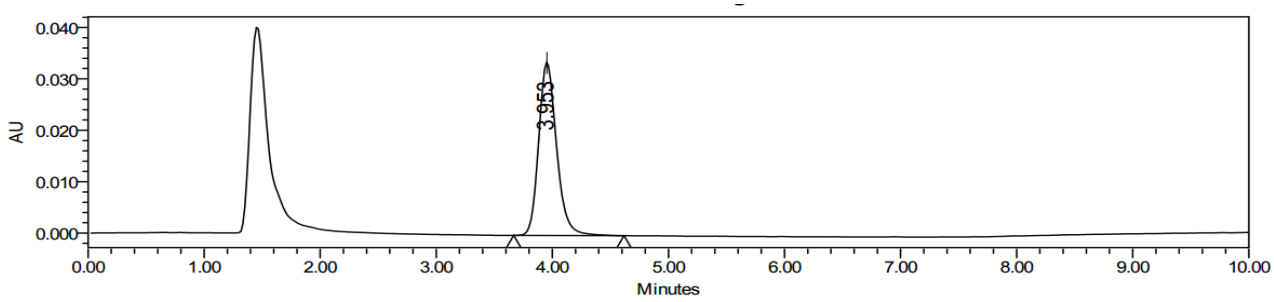


Figure 3: Typical chromatogram of Standard Solution

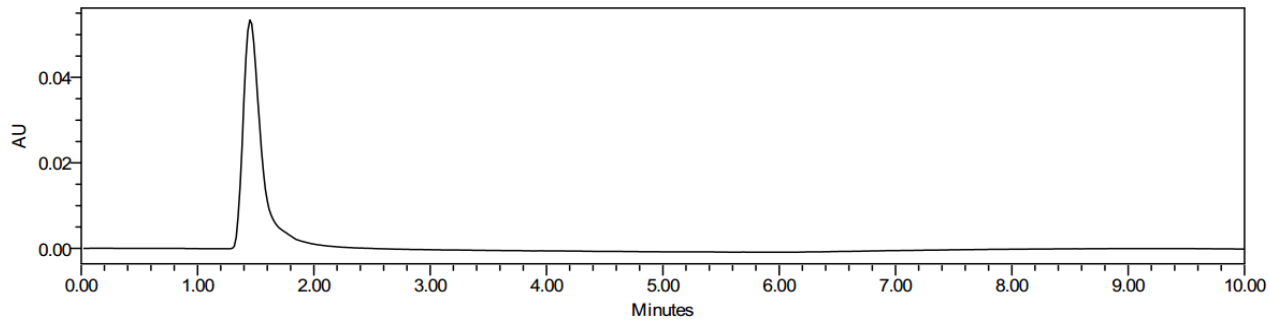


Figure 4: Typical chromatogram of 20 mg placebo

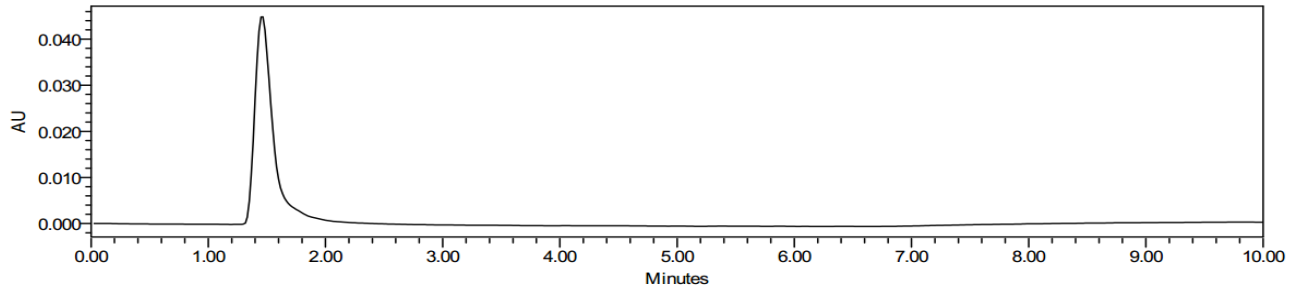


Figure 5: Typical chromatogram of 50 mg placebo

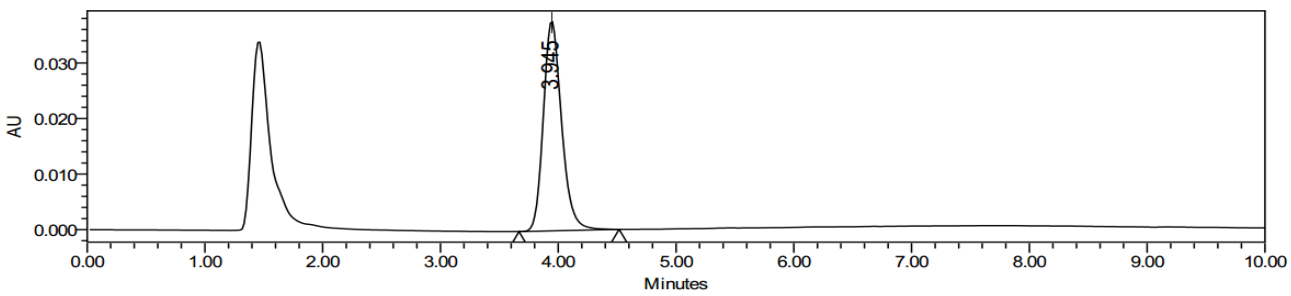


Figure 6: Typical chromatogram of 20 mg sample

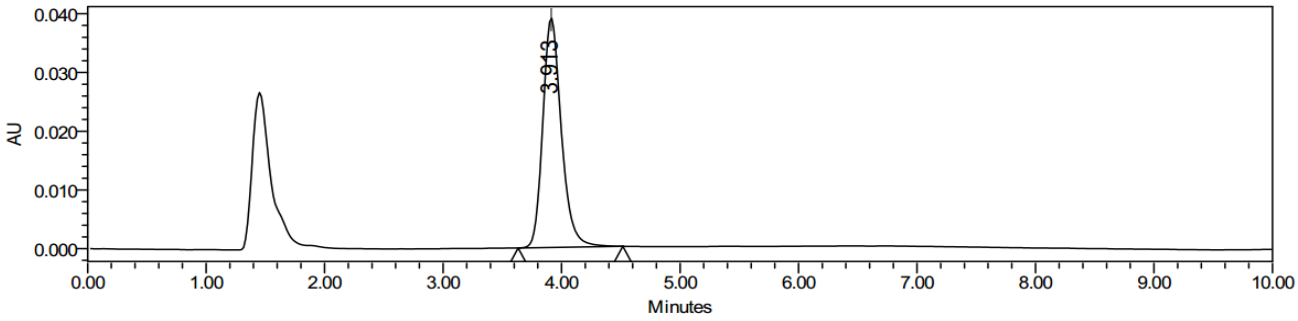


Figure 7: Typical chromatogram of 50 mg sample

RESULTS:

System suitability results

As per the test method prepared and injected system suitability standard solutions and evaluated the system Suitability parameters

Acceptance criteria	**Tailing factor NMT 2.0**	**% RSD NMT 2.0**	**Theoretical plates NLT 2000**
Results	1.2	0.6	3581

System Precision

The performance of the HPLC equipment under the chromatographic conditions is assessed by numerous injections of a homogenous standard solution. The normal solution should be injected at least six times. Six standard solution injections were made to ensure system accuracy, and the system performance was assessed in accordance with the test method requirements.

System precision results:

Parameter	**Result**	**Acceptance Criteria**
%RSD	0.6	NMT 2.0

Afatinib Standard and Sample Solution Retention Time

Name of the solution	**Retention time (Minutes)**	**Interference**
Blank	NA	No Interference
Placebo_20 mg	ND	No Interference
Placebo_50 mg	ND	No Interference
Standard	3.95	No Interference
Sample_20 mg	3.94	No Interference
Sample_50 mg	3.91	No Interference

Blank and placebo have no influence at the retention time of the analyte peak. The strategy is therefore specific.

Method Precision:

The accuracy of an analytical method describes how closely a set of measurements obtained from numerous samples of the same homogenous sample coincide. fabricated six sample preparations in accordance with the

test technique, and then injected each one into the HPLC system. figured out the percentage of drug release and RSD.

For 20 mg Strength:

Name of Sample	% of Drug release at 15 min
Preparation-1	93
Preparation-2	88
Preparation-3	96
Preparation-4	90
Preparation-5	95
Preparation-6	92
Average	**92**
% RSD	**3.3**

For 50 mg Strength:

Name of Sample	% of Drug release at 15 min
Preparation-1	99
Preparation-2	100
Preparation-3	94
Preparation-4	95
Preparation-5	98
Preparation-6	94
Average	**97**
% RSD	**2.7**

The % of drug release results should meet the specification limit. The %RSD for % of drug release results from six samples of method precision should be not more than 5.0.

Intermediate Precision:

To demonstrate the robustness of the test procedure, conduct a system-to-system, column-to-column, day-to-day, and analyst-to-analyst variability study on two HPLC systems (of the different manufacturer). These columns will be used by various analysts on various days. For each of the six formulations, the drug release percentages were computed.

Intermediate Precision results for 20 mg:

Name of Sample	% of Drug Release at 15 min
Preparation-1	92

Preparation-2	94
Preparation-3	89
Preparation-4	91
Preparation-5	93
Preparation-6	96
Average	**93**
% RSD	**2.6**

Results of Method Precision and Intermediate Precision for Cumulative Percent RSD for Percent of Drug Release for 20 mg Strength

Name	**% Assay**	
	Method precision	**Intermediate precision**
Preparation-1	93	92
Preparation-2	88	94
Preparation-3	96	89
Preparation-4	90	91
Preparation-5	95	93
Preparation-6	92	96
Overall Mean	**92**	
Overall SD	**2.6**	
Overall % RSD	**2.82**	

The experimental results met the required standards based on the aforementioned data. The technique is hence repeatable (rugged).

Linearity

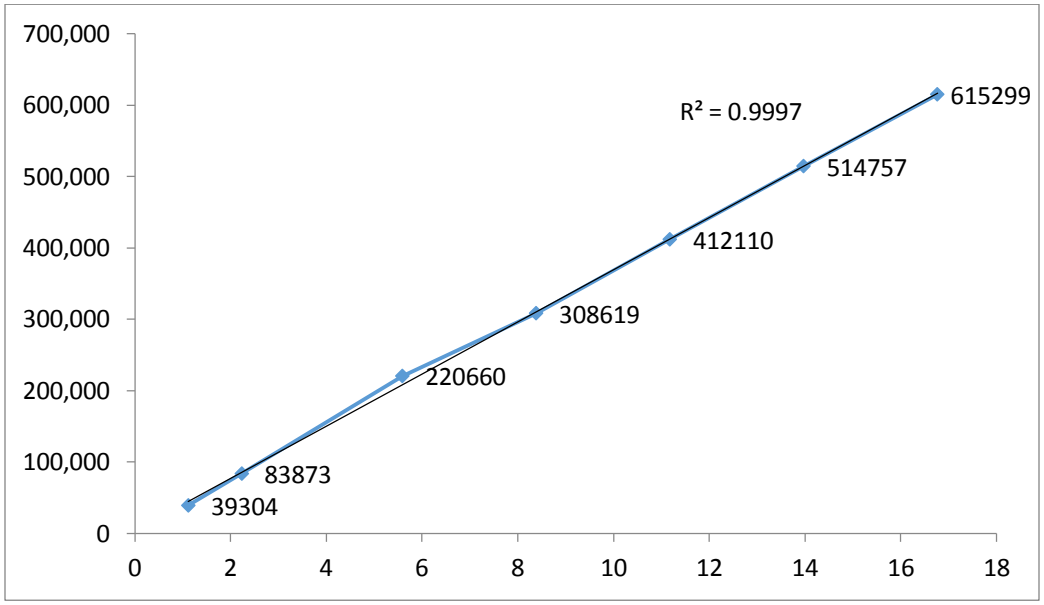
To evaluate the linearity of the method, prepare a series of standard solutions from 10% to 150 % of standard concentration and recommended not less than five levels. Perform at each level two absorbance at 10% level to 150% levels. Plot average peak response versus the concentration. Determine the correlation coefficient, regression line of the 10% to 150 % levels.

Linearity Results

Level	**Concentration in (ppm)**	**Area**
10%	1.118	39304
20%	2.236	83873
50%	5.590	220660
75%	8.385	308619
100%	11.179	412110

125%	13.974	514757
150%	16.769	615299
Correlation coefficient		**0.9997**
Regression coefficient		**0.999**

Linearity Graph of Afatinib (Peak response versus concentration)



The results met the acceptance criteria and based on experimental data the method is linear and precise at lower and higher levels.

Accuracy

The degree to which test results acquired via an analytical process are accurate in relation to the true value Analyze the system compatibility using the technique. Recovery solutions were created using stock solution and a placebo at levels ranging from 20 to 150 percent of test concentrations. The flask method was used for the accuracy investigation. Calculated the quantity found, the amount added, the mean recovery percentage, the individual recovery percentage, and the percent RSD at each level.

Preparation of Accuracy Stock Solutions :

Afatinib Dimaleate API was accurately weighed, transferred, and added to a 50 mL volumetric flask along with 25 mL of diluent. The substance was then sonicated to completely dissolve it, diluted to the required level with diluent, and thoroughly mixed.

Preparation of accuracy at 20% Level solution:

Pipetted out 10 mL of above accuracy stock solution into 50 mL volumetric flask, diluted to the volume with diluent and mixed well.

Accuracy sample solution preparation follow below mention table

Level	**Taken__ mg of Placebo Equivalent**	**Added__mL of Afatinib 20% API Stock solution**	**__ mL of dissolution medium Taken in Dissolution vessel**	**Q- Point time (min)**
Level-1 (20%)	420.4	8.0	900	15
	420.5	8.0	900	15
	420.7	8.0	900	15
Level	**Taken__ mg of Placebo Equivalent**	**Added__mL of Afatinib API Stock solution**	**__ mL of dissolution medium Taken in Dissolution vessel**	**Q Point time (min)**
Level-2 (100%)	420.4	8.0	900	15
	420.8	8.0	900	15
	420.3	8.0	900	15
Level	**Taken__ mg of Placebo Equivalent**	**__mg of Afatinib API Added**	**__ mL of dissolution medium Taken in Dissolution vessel**	**Q Point time (min)**
Level-3 (150%)	420.0	110.1	900	15
	420.6	110.2	900	15
	420.1	110.1	900	15

Accuracy results

Level	**Preparation**	**Mean % Recovery**	**Average**	**SD**	**% RSD**
Level-1	Prep-1	99.5	99.3	0.38	0.4
	Prep-2	98.9			
	Prep-3	99.6			
Level-2	Prep-1	98.5	98.5	0.55	0.6
	Prep-2	99.0			
	Prep-3	97.9			
Level-3	Prep-1	99.0	99.8	0.76	0.8
	Prep-2	100.0			
	Prep-3	100.5			

Effect of variation in flow rate and temperature, pH and Organic variation.

performed system suitability testing in accordance with the test procedure, and analysed the results. Prepared standard solutions, injected them into the HPLC system in accordance with the test procedure, and assessed the method's robustness to the following modifications.

Parameter		Main peak RT	%RSD (NMT 2.0)	Tailing Factor (NMT 2.0)	Theoretical plates
Flow	Actual (1.0 mL)	3.95	0.3	1.2	3548
	Low (0.8 mL)	5.00	0.2	1.2	3640
	High (1.2 mL)	3.21	0.6	1.2	3227
Temp	Actual (35°C)	3.95	0.3	1.2	3548
	Low (30°C)	4.54	0.4	1.2	3226
	High (40°C)	3.79	0.5	1.2	3913
Organic variation	Actual	3.95	0.3	1.2	3548
	Low	5.10	0.2	1.1	3406
	High	3.65	0.3	1.1	3031
pH variation	Actual (4.5)	3.95	0.3	1.2	3548
	Low (4.3)	3.90	0.5	1.1	3656
	High (4.7)	4.35	0.4	1.1	4022

Filter compatibility results of samples:

Prepare a standard as per methodology and test solutions (Dissolution on one dosage units by Afatinib Tablets 50 mg) as per methodology without filtration. Centrifuge one portion of test solution and filter another portion of test solution through two individual filters of 0.45 µm Nylon and PVDF. Centrifuged test solutions and filtered test solutions into the HPLC system under the test conditions. For test solutions calculate the % dissolution (% Drug release) for centrifuged and filtered test solutions against unfiltered standard as per test method.

Results for Filter study of Sample solution

Strength (50 mg)	Centrifuged samples	0.45 µm Nylon filter	Diff. from centrifuged to Nylon filter (NMT 3.0%)	0.45 µm PVDF filter	Diff. from centrifuged to PVDF filter (NMT 3.0%)
Results	98	95	3	97	1

All of the filter results were acceptable, and 0.45 PVDF filters are adequate for sample filtration. Therefore, the methodology is described for filter study.

Solution stability of standard and sample

Over the course of 24 hours at room temperature (RT) & refrigerator (RF), the solution stability of the standard solutions and the sample was established on an hourly basis. Below are the results tabulated.

Standard solution stability

Time	**Similarity factor**	**Acceptance criteria**
Initial	NA	**0.98-1.02**
STD Solution About 20 Hr_RT	1.01	
STD Solution About 20 Hr_RF	1.00	
STD Solution About 48 Hr_RT	1.01	
STD Solution About 48 Hr_RF	0.98	

Sample solution stability

According to the below mentioned data, the standard solution is stable for up to 48 hours at room temperature (RT), while the sample solutions are stable for up to 48 hours at a refrigerator (RF).

Time	**% Drug release**	**% Difference**	**Acceptance criteria**
Initial	92	NA	**NMT 2.0%**
Sample solution About 20 Hr_RT	94	2	
Sample solution About 20 Hr_RF	91	1	
Sample solution About 48 Hr_RT	94	2	
Sample solution About 48 Hr_RF	90	2	

CONCLUSION:

A simple, precise, accurate, and reproducible RP-HPLC method for quantifying afatinib in dissolution samples has been developed and validated. BOS could be completely dissolved in 900 ml of dissolution liquid after 45 minutes using equipment USP type I (Basket) at 100 rpm (pH 4.0 McIlavine buffer). Based on the findings, the developed RP-HPLC method was found to be specific, accurate, exact, rugged, robust, and linear over the concentration range. The validation parameter report met the applicable acceptance criteria. The findings were statistically supported. As a result, the developed approach can be used for routine quality control analysis and Afatinib dissolution studies in bulk and pharmaceutical dose form.

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