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Development and validation of a stability indicating uplc method for determination of erlotinib in pharmaceutical formulations

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ABSTRACT

Simple, rapid, sensitive, accurate, robust & rugged stability indicating analytical method for determination of Erlotinib in pharmaceutical formulations is developed and validated by using UPLC & applied the developed and validated method for determining the assay of Erlotinib in tablets (Tarceva), as there is no official monograph & no analytical method by UPLC. Chromatography was performed with mobile phase containing potassium dihydrogen ortho phosphate, added 1mL of triethylamine and adjusted to pH 2.4 with orthophosphoric acid , with a flow rate of 0.3mL/min, C-18 column & UV detection at 225nm.The method was validated for linearity, accuracy, ruggedness, robustness, precision & bench top stability of sample & standard solution. Erlotinib tablets were subjected to different stress conditions like acid, alkali, peroxide, thermal, water & UV studies and checked for its specificity, degradation & stability. The developed method was very rapid with a run time of 3 min, accurate, robust, rugged and stable.

Keywords: Erlotinib, Assay method, UPLC, Stability indicating method.

INTRODUCTION

Ultra performance liquid chromatography TM (UPLC) takes advantage of technological strides made in particle chemistry performance, system optimization, detector design, and data processing and control. Using sub-2 mm particles and mobile phases at high linear velocities, and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity, and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. [1]

According to an FDA guidance document, a stability-indicating method is “a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities.”[2]

Erlotinib is white to off-white powder , designated chemically as N-(3-Ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine hydrochloride with an empirical formula of $C_{22}H_{23}N_3O_4 \cdot HCl$ and a molecular weight of 429.90 (Fig.1). Erlotinib is very slightly soluble in methanol, insoluble in acetonitrile, acetone, ethyl acetate, hexane &

aqueous solubility of erlotinib is dependent on pH with increased solubility at a pH of less than 5 due to protonation of the secondary amine. It has a pKa of 5.42 at 25^{o}C.[3-5]

Tarceva (erlotinib) is a Human Epidermal Growth Factor Receptor Type 1/Epidermal Growth Factor Receptor (HER1/EGFR) tyrosine kinase inhibitor. While the complete mechanism of action is not entirely understood, the HER1 and EGFR receptors are directly involved in inter-cellular signalling in systems governing cell division and proliferation. By inhibiting the function of these receptors, which are highly active and often over-expressed in rapidly dividing tumor cells, Tarceva is thought to limit tumor cells' ability to divide and metastasize, and may help to initiate pathways of apoptotic cell death.[6-8]

A few methods for the determination of Erlotinib in pharmaceutical formulations by HPLC, HPTLC and UV appear in literature. So far no systematic UPLC method has been reported for determination of Erlotinib in pharmaceutical formulations. This paper reports a rapid and sensitive UPLC method with UV detection, useful for routine quality control of Erlotinib Hydrochloride in pharmaceutical formulations. The method was validated by parameters such as linearity, accuracy, precision, robustness, ruggedness, sample and standard solution stability and forced degradation studies.[9]

MATERIALS AND METHODS

Reagents:
HPLC grade Acetonitrile (HPLC Grade, Merck), Potassium dihydrogen orthophosphate (AR, Rankem), Hydrochloric Acid (AR, Rankem) Sodium hydroxide (AR, Rankem), Hydrogen peroxide (AR, Rankem), Ortho phosphoric acid (AR, Rankem),Water (Milli Q water),. Erlotinib pure drug substance was kindly supplied by Strides Arcolabs Limited, India. Ingredients used for placebo were lactose monohydrate, hypromellose, hydroxypropyl cellulose, magnesium stearate, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate and titanium dioxide.

Instrumentation:
A liquid chromatograph (Waters Acquity) system equipped with an injection valve (Rheodyne), & PDA detector. The UPLC system was well equipped with Empower 2 software for data processing. Other instruments like Sartorius Analytical Balance, Metrohm pH Meter and Biotechnics sonicator were used in sample and standard preparations and for forced degradation studies.

Chromatographic conditions:
The analytical column used was Waters HSS, C-18, 100X2.1; 1.8µm .The mobile phase was potassium dihydrogen ortho phosphate, adjusted to pH 2.4 with ortho phosphoric acid. It has a flow rate of 0.3mL/min, injection volume of 1µL with ambient column oven temperature and sample tray temperature with isocratic elution & UV detection at 225nm & a run time of 3 min.

Standard, sample, mobile phase and diluent preparation:
Diluent: Methanol & Mobile phase are used as diluents.

Preparation of mobile phase: Accurately weigh and transfer about 3.5 grams of Potassium di-hydrogen phosphate in 1000 mL of purified water, added 1.0 mL of Triethylamine and adjusted the pH to 2.4 (±0.05) with dilute orthophosphoric acid and sonicated to degas.

Preparation of standard solution:
Accurately weighed and transferred 50mg of Erlotinib in to a 100mL volumetric flask and added 70mL of diluent.Sonicated for 5 min and made up to the mark with diluent.Transferred 4mL of above solution to 20mL volumetric flask and made up to volume with diluent.Filtered with 0.45µm PFTE filter.

Preparation of Test solution:
Weighed & crushed 5 tablets and transferred in to a 100mL volumetric flask and added 75mL of methanol.Sonicated in cold water for 20minutes with intermittent shaking.Allowed it to cool to room temperature and diluted to volume with diluent.Filtered atleast 10mL of the above solution with 0.45µm PTFE filter and transfered 4mL of filtered solution to 200mL volumetric flask and made up to volume with mobile phase.

RESULTS AND DISCUSSION

Specificity:
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.[10] Specificity was demonstrated by injecting a blank, placebo and standard solution. No interference was seen at the retention time of analyte. The specificity was also demonstrated by induced degradation of Erlotinib formulation and placebo samples to acid degradation, alkali degradation, peroxide degradation, thermal degradation, water degradation, U.V. degradation. Purity angle is less than purity threshold for all the stress conditions. The results are tabulated in Table No.:1.Figures 4-9 represents different stress conditions.

System suitability Testing:
System suitability testing is used to verify that the reproducibility of the system is adequate for the analysis to be performed. System suitability is done by preparing and injecting the standard solution 5 times and calculating its RSD. Other parameters like tailing and theoretical plates should also be taken in to consideration. Results are tabulated in Table No.:2

Linearity:
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample [10]. The linearity of the test method was performed by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis from 40% to 160% of test concentration and calculated the correlation coefficient, it was found to be 0.999.The results are tabulated in Table No.:3 and the graphs are represented as Fig No.:10.

Limit of detection (LOD) and limit of quantification (LOQ):
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. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [10]. Calculated the LOD & LOQ, with the calculations obtained from evaluation of the calibration curve of the linearity. LOD and LOQ values are less than the minimum linearity concentration.
The calculations and results are tabulated in Table. No.:4

Bench top stability of standard & test preparation:
Performed the assay of Erlotinib as per the test method in duplicate and kept the standard and test solutions on the bench top for 48 Hrs. Injected at initial, 24 Hrs and 48 Hrs. Calculated the difference between initial and bench top stability samples for % assay of Erlotinib for test solutions and similarity factor for standard solutions were found to be within limits. The results are tabulated in Table No.:5

Accuracy:
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found [10]. Performed the accuracy of test method using Erlotinib placebo at 50%, 100%, 125%, spike levels. The % assay at each spike level was found to be between 95.0-105.0% of the labeled amount. The results are tabulated in Table No.:6

Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility [10].

Method precision:
Determined the precision of the test method by preparing & injecting 6 test solutions of Erlotinib formulations in to the chromatograph and recorded the results. The average % assay was found to be 100.4 with % RSD of 0.62. The results are tabulated in Table No.:7

Intermediate precision:
Performed the assay of Erlotinib by following the same procedure as that of Method precision but on a different day and by a different analyst. The average % assay was found to be 99.4% with % RSD of 0.39.Overall RSD when compared with Method precision is 0.73. The results are tabulated in Table No.:8&9

Robustness:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [10]. Robustness was performed by injecting the Erlotinib standard solution in to the UPLC by altering the Flow rate, Column oven temperature and also by changing the pH of the buffer & composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in Table No.:10

Table No.: 1

ERLOTINIB FORCED DEGRADATION		
Stress Condition	Purity Angle	Purity Threshhold
Acid Stress	0.115	0.285
Alkali Stress	0.130	0.275
Peroxide Stress	0.138	0.285
Water Stress	0.125	0.267
Heat Stress	0.145	0.285
U.V. Stress	0.132	0.265
Acceptance Criteria	Peak Purity shall pass	

Table No.:2

ERLOTINIB SYSTEM SUITABILITY									
Injection No.	1	2	3	4	5	Mean	STDEV	RSD	*Limits*
Standard Area	2305687	2302824	2311478	2300543	2283295	2300765	10589	0.5	RSD NMT 2.0%
Theoretical Plates	7818	7835	7825	7826	7829	7827	6.19	0.1	NLT 2000
USP tailing	1.54	1.54	1.54	1.54	1.53	1.54	0.00	0.3	*NMT 2.0*
RT	1.259	1.260	1.263	1.265	1.267	1.263	0.00	0.3	

Table No.:3

ERLOTINIB-LINEARITY						
Run	% Conc.	Conc. Of Erlotinib (µg/mL)	Area of Erlotinib	Slope	Y-intercept	R^2
1	40%	50.00	937722	18454.9	27292.95	0.999
	80%	100.00	1908256			
	100%	125.00	2295800			
	120%	150.00	2819056			
	160%	200.00	3709937			
2	40%	50.00	942173	18555.3	25535.15	0.999
	80%	100.00	1908189			
	100%	125.00	2301865			
	120%	150.00	2852614			
	160%	200.00	3719921			
3	40%	50.00	943469	18463.6	31258.15	0.999
	80%	100.00	1902911			
	100%	125.00	2306901			
	120%	150.00	2831549			
	160%	200.00	3711182			
Average				18491.26067	28028.75	0.999
Standard Deviation				55.66	2931.59	0.00
Acceptance criteria: Coefficient of correlation shall be NLT 0.999						

Calculation:
%Assay:

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{4}{20} \times \frac{100}{Wt} \times \frac{200}{4} \times \frac{P}{100} \times \frac{100}{L} \times 100 =$$

At=Area of test solution, P=Potency of Erlotinib Working Std.on as is basis, As=Area of standard solution Avg. Wt. =Avg. Wt. of 20 tablets, Ws=Weight of standard taken, LC=Label claim of the tablet as Erlotinib, Wt=Weight of tablets

Table No.:4

ERLOTINIB- LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)				
S.No.	Injection No.	Slope	Y-Intercept	R^2
1	Inj-1	18454.90	27292.95	0.999
2	Inj-2	18555.34	25535.15	0.999
3	Inj-3	18463.55	31258.15	0.998
Average		18491.2633	28028.7500	0.9987
STDEV		55.660	2931.592	0.001
LOD=3.3 x σ/S		LOQ=10 x σ/S		
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOD	0.5	ppm		
LOQ	1.6	ppm		
Acceptance Criteria: LOD & LOQ values shall be less than the minimum linearity concentration				

Table No.:5

ERLOTINIB BENCH TOP STABILITY OF STANDARD SOLUTION						
Time(Hrs)	Day	Std. Wt.	Response	Fresh Std Wt.	Response of fresh std.	Similarity Factor
Initial	Initial	50.30	2300765			
24 Hrs	Day-1	50.30	2311082	50.15	2316978	1.00
48 Hrs	Day-2	50.30	229288	50.42	2268919	0.99
ERLOTINIB BENCH TOP STABILITY OF TEST SOLUTION-1						
Time(Hrs)	Day	Weight(mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	1252.65	2337254	101.29	NA	
24 Hrs	Day-1	1252.65	2331881	100.6	0.7	
48 Hrs	Day-2	1252.65	2305445	101.01	0.3	
ERLOTINIB BENCH TOP STABILITY OF TEST SOLUTION-2						
Time(Hrs)	Day	Weight(mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	1246.45	2321427	100.6	NA	
24 Hrs	Day-1	1246.45	2320794	100.12	0.5	
48 Hrs	Day-2	1246.45	2327728	101.99	1.4	

Table No.:6

Standard Preparation	50.43	mg	4	Potency	99.0
	100		20		
Sample Preparation	Wt. of sample taken in mg		4	Label Claim	100
	100		200		
Standard Area		2316978	Average Wt. in mg		250

ERLOTINIB-ACCURACY				
Spike level	Wt. of sample taken in mg	Sample area	% Recovery	Average
50%_01	625.00	1159290	99.9	99.8
50%_02	625.00	1155954	99.6	
50%_03	625.00	1158198	99.8	
100%_01	1250.00	2292178	98.8	98.3
100%_02	1250.00	2281190	98.3	
100%_03	1250.00	2272375	97.9	
150%_01	1875.00	3400552	97.7	97.9
150%_02	1875.00	3406155	97.9	
150%_03	1875.00	3411601	98.0	
Acceptance criteria: % Average recovery shall be between 95.0% -105.0%				

Table No.: 7

ERLOTINIB ANALYTICAL MEHTOD VALIDATION-ASSAY								
Method Parameter			METHOD PRECISION					
Std. wt. & Dilution	50.33	4	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	4	Label claim (mg)	100
	100	20	250		100	200	Potency (%)	99
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample	Assay %	Average (%)	STDEV	% RSD
1	2310915	1.54	1250.00	2337254	101.23	100.71	0.51552	0.51
2	2290693	1.54	1250.00	2321427	100.55			
3	2300684	1.54	1250.00	2317128	100.36			
4	2300777	1.54	1250.00	2341249	101.41			
5	2300755	1.54	1250.00	2324067	100.66			
			1250.00	2310208	100.06			
Average	2300765	1.54	1250.00	2325222	100.71			
STDEV	7149.73	0.00		% RSD of 6 replicate injections is not more than 2				
%RSD	0.31	0.00						

Table No.:8

ERLOTINIB ANALYTICAL MEHTOD VALIDATION-ASSAY								
Method Parameter			INTERMEDIATE PRECISION					
Std. wt. & Dilution	50.25	5	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	5	Label claim (mg)	100
	100	20	250		100	200	Potency (%)	99.0
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample	Assay %	Average (%)	STDEV	% RSD
1	2315498	1.52	1250.00	2303175	98.90	99.29	0.373	0.38
2	2302693	1.52	1250.00	2318575	99.56			
3	2314434	1.52	1250.00	2314650	99.40			
4	2321577	1.52	1250.00	2305262	98.99			
5	2330688	1.52	1250.00	2325271	99.85			
			1250.00	2306776	99.06			
Average	2316978	2	1250	2312285	99.29			
STDEV	10269.35	0.00	Limits	% RSD of 6 replicate injections is not more than 2				
%RSD	0.4	0.0						

Table No.:9

ERLOTINIB ANALYTICAL MEHTOD VALIDATION-ASSAY							
Method Parameter		**METHOD & INTERMEDIATE PRECISION COMBINEDLY**					
Method Precision		Intermediate Precision					
S.No.	% Drug content	S.No.	% Drug content	Difference	Average of both Method & Intermediate precision	STDEV of both Method & Intermediate precision	%RSD of both Method & Intermediate precision
1	101.23	1	98.9	2.3	100.0	0.856	0.86
2	100.55	2	99.6	1.0			
3	100.36	3	99.4	1.0			
4	101.41	4	99.0	2.4			
5	100.66	5	99.9	0.8			
6	100.06	6	99.1	1.0			
Limits: Overall RSD when compared with Method precision should be not more than 2%.							

Table No.:10

ERLOTINIB ANALYTICAL MEHTOD VALIDATION-ASSAY					
Method Parameter			ROBUSTNESS		
Change in Flow Rate(0.25mL/min)			Change in Flow Rate(0.35mL/min)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	2743760	1.55	1	1973875	1.49
2	2774673	1.55	2	1943344	1.49
3	2740829	1.55	3	1960245	1.49
4	2732432	1.55	4	1952056	1.49
5	2734277	1.55	5	1958542	1.49
Average	2745194	1.55	Average	1957612	1.49
STDEV	17118.49	0.00	STDEV	11255.31	0.00
%RSD	0.62	0.0	%RSD	0.57	0.0
Change in pH of Mobile Phase (1.6)			Change in pH of Mobile Phase (2.0)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	2271424	1.49	1	2263481	1.53
2	2252217	1.49	2	2258739	1.53
3	2249439	1.49	3	2276006	1.53
4	2244184	1.49	4	2272593	1.53
5	2241573	1.48	5	2276184	1.53
Average	2251767	1.49	Average	2269401	1.53
STDEV	11762.64	0.00	STDEV	7882.71	0.00
%RSD	0.52	0.3	%RSD	0.35	0.0
Change in Org Phase Comp.(90%)			Change in Org Phase Comp.(110%)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	2311223	1.43	1	2265737	1.53
2	2313683	1.43	2	2269570	1.53
3	2305552	1.43	3	2290266	1.53
4	2315524	1.43	4	2291368	1.53
5	2306395	1.43	5	2290691	1.53
Average	2310475	1.43	Average	2281526	1.53
STDEV	4393.90	0.00	STDEV	12742.53	0.00
%RSD	0.19	0.0	%RSD	0.56	0.00

Fig. No.1: Erlotinib Hydrochloride

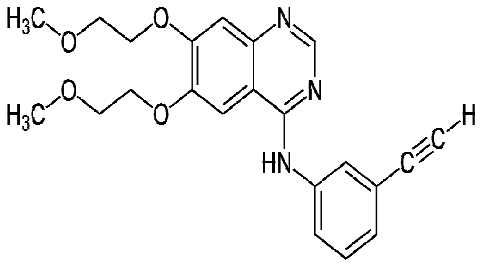


Fig. No.2: Blank-Diluent

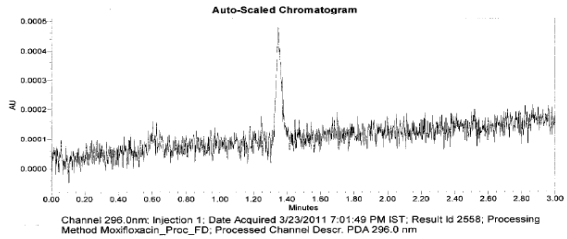


Fig.No.3: Standard

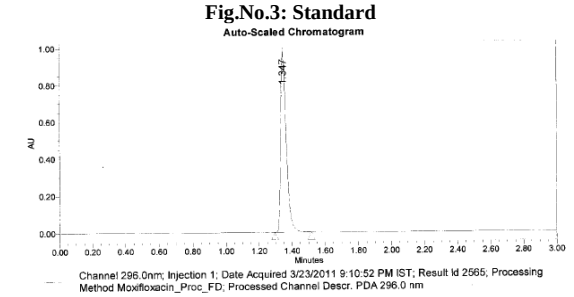


Fig.No.4: Acid Stressed Sample

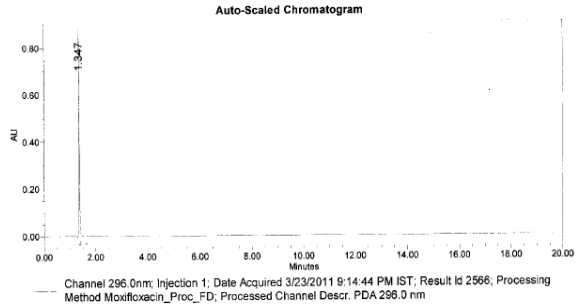


Fig.No.5: Alkali Stressed Sample

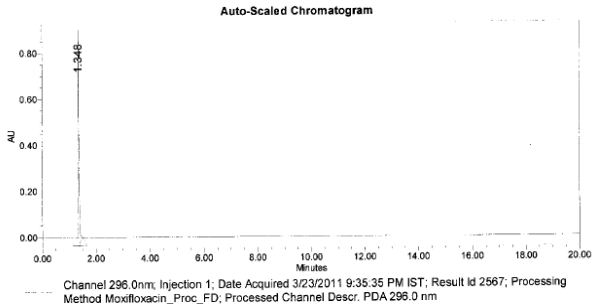


Fig.No.6: Peroxide Stressed Sample

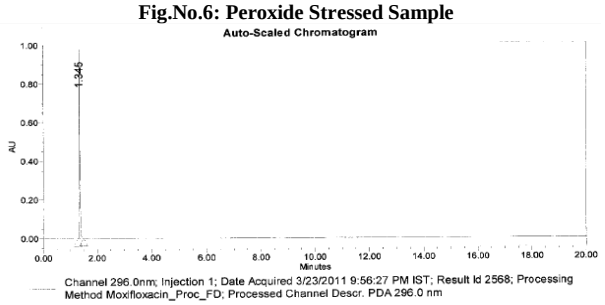


Fig.No.7: Water Stressed Sample

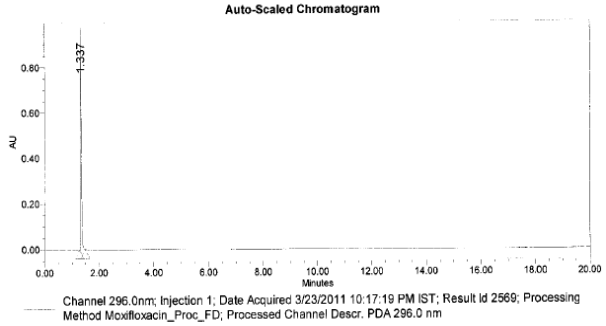


Fig.No.8: Heat Stressed Sample

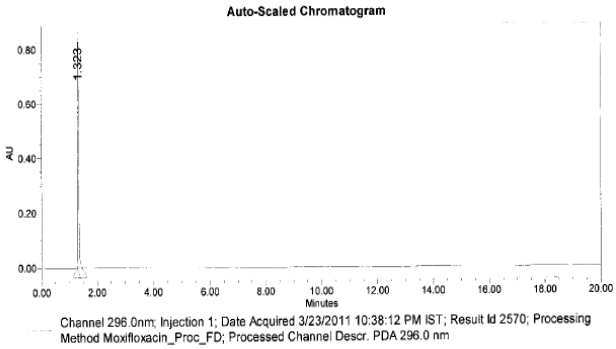


Fig.No.9: UV Stressed Sample

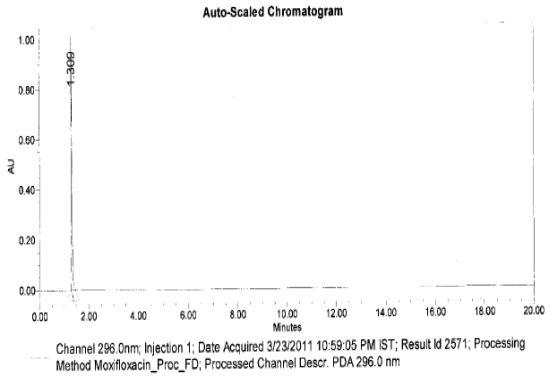
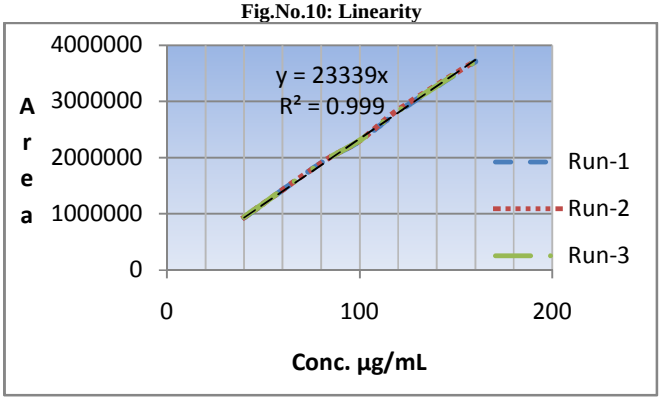


Fig.No.10: Linearity



CONCLUSION

The reported UPLC method was proved to be simple, rapid with a runtime of 3 min & reproducible. The validation data indicates good specificity, precision, accuracy & reliability of the method. The developed method has many advantages like isocratic mode of elution, easy sample preparation, short run time and can be used for routine quality control analysis of Erlotinib formulations.

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