DEVELOPMENT & VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF ERLOTINIB AND ITS IMPURITIES IN PHARMACEUTICAL DOSAGE FORM

Y.S.R.V.S Jogarao, Ponnuri Bharath, Dr.V.Siva Ramakrishna, Dr.D.Ramachandran*

Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P, India

Corresponding author E-mail: dittakavirc@gmail.com

ABSTRACT

The analysis of improved RP-HPLC method for the separation and quantification of Erlotinib and its impurities are described. Samples are analysed by means of reverse phase (RP-HPLC) using an Kromasil C18, 250 x 4.6 mm, 5µm and the mobile phase consists of two channels A and B. Channel-A: pH 2.80 phosphate buffer and Channel-B: Acetonitrile. The flow rate is 1.2 ml/min. The column temperature was maintained at 50°C and sample temperature was maintained at 5°C, injection volume 10µL and wavelength fixed at 248nm UV-detection. The developed LC method was validated with respect to specificity, precision, linearity, ruggedness and robustness. Validation study compared as per ICH guideline.

Key words: Erlotinib, estimation of related substances, liquid chromatography.

1.0 Introduction

Erlotinib (ERL), chemically known as N-(3-ethynylphenyl)-6, 7-bis (2-methoxyetho -xy) quinazolin-4-amine. Erlotinib is an epidermal growth factor receptor inhibitor (EGFR inhibitor) and used to treat non small cell lung cancer (NSCLC)), the oral epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitor (TKI). Erlotinib is an established second-line treatment for advanced NSCLC [1-2]. The molecule structure is shown in **Figure 1.**

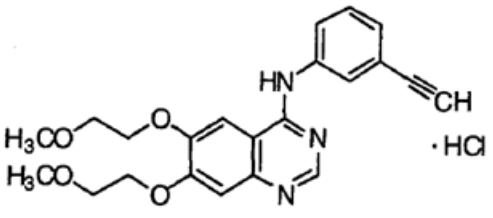


Figure 1. Chemical structure of Erlotinib

A few analytical methods have been reported for quantitative estimationof Erlotinib in pharmaceutical formulations. Simple and selective method for the determination of various tyrosine kinase inhibitors used in the clinical setting by LC-MS [3]. Development and validation of the UV Spectrophotometric method for the determination of Erlotinib in tablet formulation [4]. Spectrophotometric method for the determination of Erlotinib in pure and pharmaceutical dosage form [5]. Development and validation of high performance liquid chromatographic method for the determination of Erlotinib [6]. Spectroscopic studies on the interaction between Erlotinib hydrochloride and bovine serum albumin [7]. Extractive colorimetric method development and validation for Erlotinib in bulk and tablet dosage form [8].

The objective of the present work is to develop a stability indicating HPLC method and validated as per ICH [9] and USP validation guidelines.

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and formulations is one of the most challenging tasks. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products. For these reasons, all major international pharmacopoeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product.

2.0 Experimental

Reagents and chemicals

Potassium dihydrogen orthophosphate, Triethylamine, Orthophosphoric acid, Acetonitrile and methanol was procured from Merck. Water (Milli-Q). All chemicals were of an analytical grade and used as received.

Instrumentation

Chromatographic separation was achieved by using an Waters e2695, Empower^3 software using, Kromasil C18, 250 x 4.6 mm, 5µm column and the mobile phase consists of two channels A and B. Channel-A: pH 2.80 phosphate buffer and Channel-B: Acetonitrile. The flow rate is 1.2 ml/min. The column temperature was maintained at 50°C and sample temperature was maintained at 5°C, injection volume 10µL and wavelength fixed at 248nm UV-detection. Retention times of impurities were 4.75 for impurity-A, 24.82 for Impurity-B, 7.50 for Impurity-C and 26.33 for Erlotinib.

Preparation of solutions:

Preparation of mobile phase-A:

Weighed and transferred 2.72 g of potassium dihydrogen orthophosphate into a 1000 mL of water and mixed well. Added 1.0 mL of Triethylamine and adjusted the pH to 2.8 ± 0.02 with phosphoric acid and mixed well. Filtered through 0.45 µm membrane filtered and sonicated to degas.

Preparation of mobile phase-B:

Use Acetonitrile (100%) as Mobile phase-B.

Preparation of diluent:

Prepared a mixture of 500 mL of water and 500 mL of methanol in the ratio of 50:50 (%v/v).

Preparation of standard stock solution:

Weighed accurately 22.35 mg of Erlotinib hydrochloride working standard (Equivalent 20 mg of Erlotinib) and transferred into 100 mL volumetric flask, added 60 mL of diluent and sonicated to dissolved, diluted to volume with diluent and mixed well.

Preparation of standard solution:

Transferred 1.0 mL of standard stock solution into 100 mL volumetric flask, diluted to volume with diluent and mixed well.

Preparation of placebo solution:

Weighed and transferred 218.24 mg of Erlotinib Placebo powder (equivalent to 100 mg of Erlotinib) into 100 mL volumetric flask, added 60 mL of the diluent and sonicated to dissolved for 20 minutes, with intermediate shaking the flask, and kept aside to cool room temperature and diluted to volume with the diluent and mixed well. Filtered through 0.45 µm PVDF syringe filter.

Preparation of test solution:

Weighed and transferred 307.30 mg of Erlotinib sample powder (equivalent to 100 mg of Erlotinib) into 100 mL volumetric flask, added 60 mL of the diluent and sonicated to dissolved for 20 minutes, with intermediate shaking the flask, and kept aside to cool room temperature and diluted to volume with the diluent and mixed well. Filtered through 0.45 µm PVDF syringe filter.

3.0 Results and Discussion

3.1 Method optimization parameters

An understanding of the nature of API (functionality, acidity, or basicity), the synthetic process, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting.

3.2 Selection of wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 248 nm from the absorption spectrum.

3.3. Selection of stationary phase

Proper selection of the stationary phase depends up on the nature of the sample and chemical profile. The drug selected for the present study was polar compound and could be separated either by normal phase chromatography or reverse phase chromatography. From literature survey, it was found that different C18 columns could be appropriately used for the separation of related substances for Erlotinib.

3.4. Selection of mobile phase

Different mobile phases were employed to develop a suitable LC method for the quantitative determination of impurities in Erlotinib. Different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Erlotinib.

Poor peak shape and resolution was observed when Hypersil BDS C18 (250mm x 4.6mm, 5µ) and gradient mobile phase programmed of Mobile Phase: A 0.1% OPA in water and Mobile Phase: B Acetonitrile. There was no proper resolution of impurities and analyte peak and efficiency of the peak is also not achieved and peak interferences are present.

In second attempt made using Kromasil C18, 250 x 4.6 mm, 5µm column, and gradient mobile phase programmed of mobile Phase: A pH 2.80 buffer and mobile Phase: B Acetonitrile. The resolution of both drug and impurities was achieved. These chromatographic conditions were selected for validation studies.

4.0 Method Validation

4.1 Specificity

Specificity was demonstrated by injected blank solution, placebo solution, standard solution, sample solution, spiked sample and individual impurities and analyzed as per the test method.

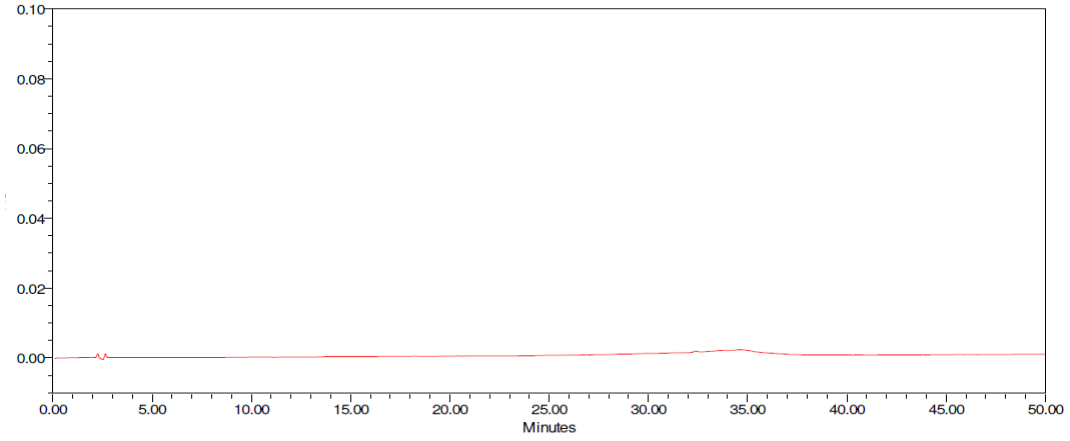


Figure 2. typical chromatogram of Blank

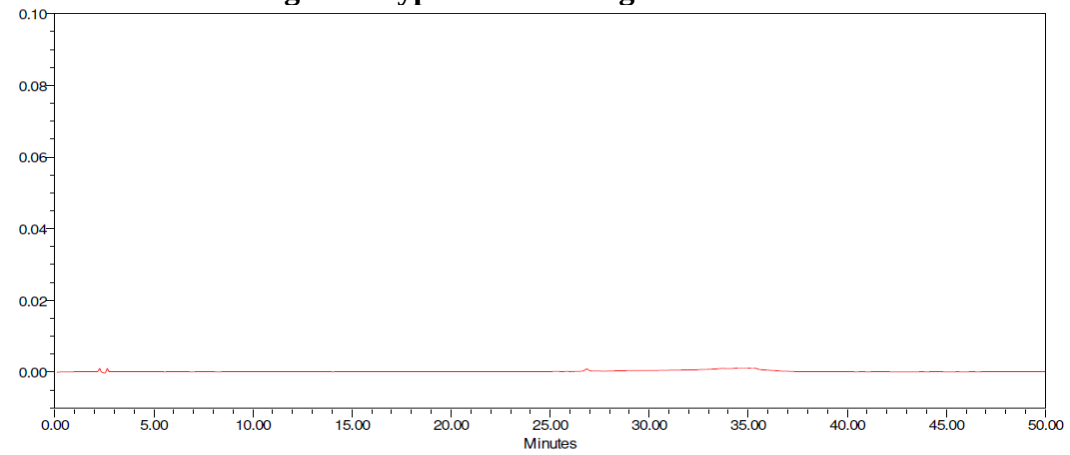


Figure 3. typical chromatogram Diluted Standard

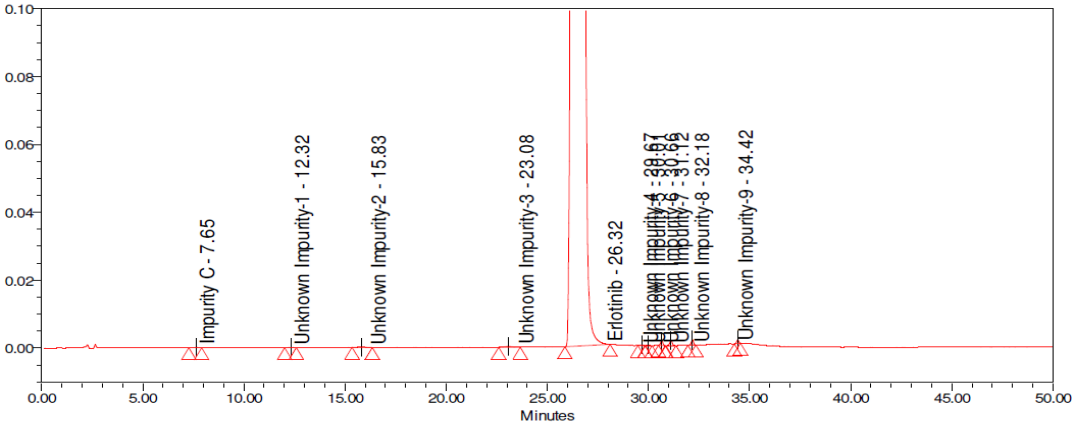


Figure 4. typical chromatogram as such Sample

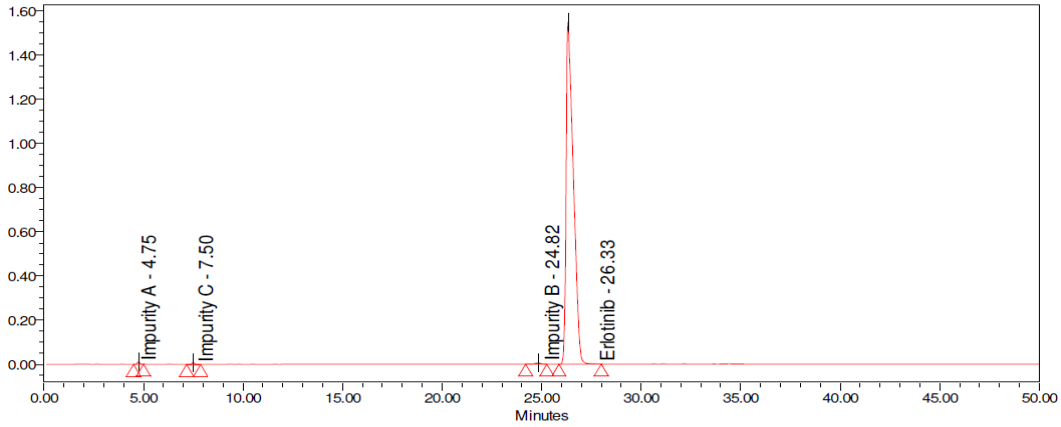


Figure 5. Typical chromatogram Spiked Sample

Table 1. Impurity interference data (Specificity results)

Peak Name	Retention Time	Blank	Placebo
Blank	ND	**NA**	**NA**
Placebo	ND	**NA**	**NA**
Impurity-A	4.75	No	No
Impurity-B	24.82	No	No
Impurity-C	7.50	No	No
Erlotinib	26.33	No	No

It was observed that known impurities are not co eluting with each other and main analyte peak.

4.2 Precision

4.2.1 System Precision:

System precision

System precision was demonstrated by prepared standard solution as per the test method and injected for six times into HPLC system. The retention time and area response of analyte peak were recorded.

Table 2. System Precision data for Erlotinib

S.No.	Area response	Retention time
1	81467	27.29
2	82133	27.30
3	82649	27.30
4	84004	27.27
5	82452	27.27
6	83665	27.27
Average	**82728**	**27.28**
STDV	**952.125**	**0.015**
% RSD	**1.2**	**0.1**

The %RSD of peak area for Erlotinib was found to be 1.2% which is below 5.0% indicates that the system gives precise result.

4.2.2 Method Precision

Method precision was demonstrated by prepared six samples by spiking of impurities at specification level and analyzed as per the test method. The samples were prepared as per the method and the result for precision study is tabulated in **Table 3.**

Table 3. Results of method precision

S.No.	Sample Details	Impurity (% Recovery)		
		Impurity-A	Impurity-B	Impurity-C
1	Method Precision Prep-1	109.0	89.9	100.0
2	Method Precision Prep-2	109.0	91.1	100.5
3	Method Precision Prep-3	108.0	90.5	100.5
4	Method Precision Prep-4	108.5	90.5	100.0
5	Method Precision Prep-5	108.5	92.2	99.5
6	Method Precision Prep-6	108.5	89.9	99.0
Avg.		108.6	90.7	99.9
Std. Dev.		0.3764	0.8681	0.5845
%RSD		0.3	1.0	0.6

The results were well within the limits. From the above results, it is concluded that method is precise.

4.3 Limit of detection (LOD) & Limit of Quantitation (LOQ)

Limit of detection: A solution containing 0.04 µg/ml of Erlotinib standard, 0.02 µg/ml of impurity-A, 0.03 µg/ml of impurity-B and 0.01 µg/ml of impurity-C was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections.

Limit of Quantitation: A solution containing 0.12 µg/ml of Erlotinib standard, 0.05 µg/ml of impurity-A, 0.10 µg/ml of impurity-B and 0.03 µg/ml of impurity-C was injected six times. The worst found signal to noise ratio for each peak was greater than 10 in each injection. All the peaks were detected in all the six injections.

Table 4. LOQ for Erlotinib and impurities

S.No.	Name of the solution	Impurity-A	Impurity-B	Impurity-C	Erlotinib
1	LOQ precision-1	2804	8184	3248	5754
2	LOQ precision-2	2729	6994	3306	5457
3	LOQ precision-3	2872	6942	3319	5833
4	LOQ precision-4	2640	6518	3022	6133
5	LOQ precision-5	2607	6518	3384	5865
6	LOQ precision-6	2772	6660	3557	5464
Avg.		2737	6969	3306	5751
Std.Dev.		100.311	629.263	175.085	258.634

%RSD	3.7	9.0	5.3	4.5
S/N	10.33	9.85	10.75	12.62

The limit of quantitation and limit of detection values obtained for each impurity and Erlotinib are within the acceptance criteria.

4.4 Linearity

The linearity of detector response for analytes was demonstrated by preparing solutions over the range of 0.1%, 0.5% & 1.0% of specification limit with respect to sample concentration. These solutions were injected into the HPLC system and the responses of the same were recorded. A plot of concentration vs. peak area was done. The Coefficient of determination between concentration and response was evaluated. The observations are tabulated below.

Table 5. Linearity for Impurity-A

S.No	Linearity Level	Concentration in ppm	Area response
1	0.1% level	1.10	114024
2	0.5% level	5.39	446417
3	1.0% level	8.69	731267
Correlation coefficient (r)			0.9995
Square root of Correlation coefficient (r^2)			0.9991
Slope			81113.55
Intercept			20007.64
RRF			0.99

Figure 6. Linearity graph of Impurity-A

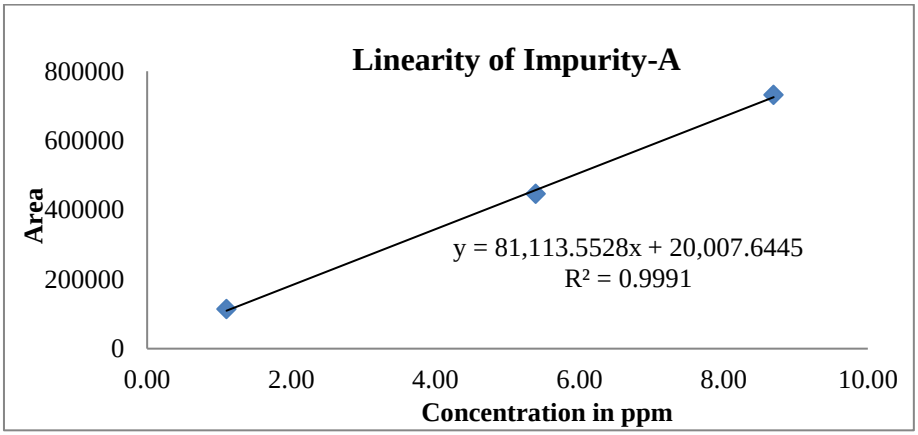


Table 6. Linearity for Impurity-B

S.No	Linearity Level	Concentration in ppm	Area response
1	0.1%	1.00	136038
2	0.5%	5.04	607826
3	1.0%	10.03	1208831
Correlation coefficient (r)			1.0000
Square root of Correlation coefficient (r^2)			0.9999
Slope			118802.30
Intercept			14325.61
RRF			1.45

Figure 7. Linearity graph of Impurity-B

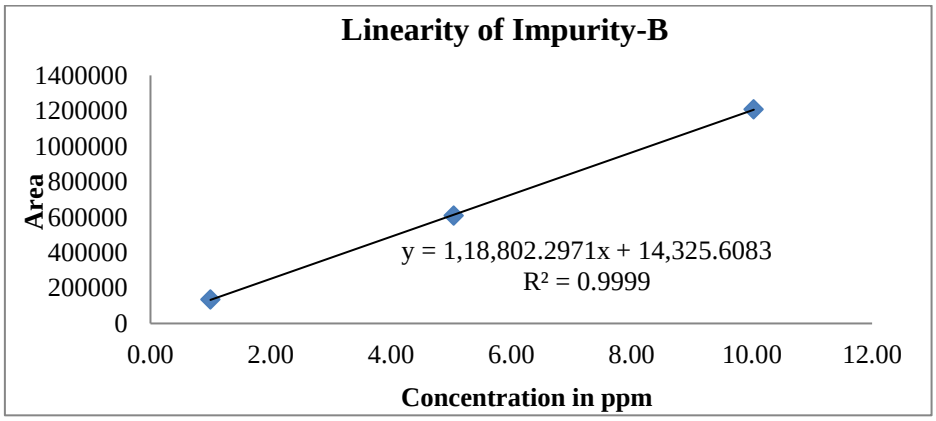


Table 7. Linearity Impurity-C

S.No	Linearity Level	Concentration in ppm	Area response
1	0.1%	1.01	84643
2	0.5%	5.04	412763
3	1.0%	10.09	721387
Correlation coefficient (r)			0.9967
Square root of Correlation coefficient (r^2)			0.9933
Slope			69773.98
Intercept			30907.13
RRF			1.82

Figure 8. Linearity graph of Impurity-C

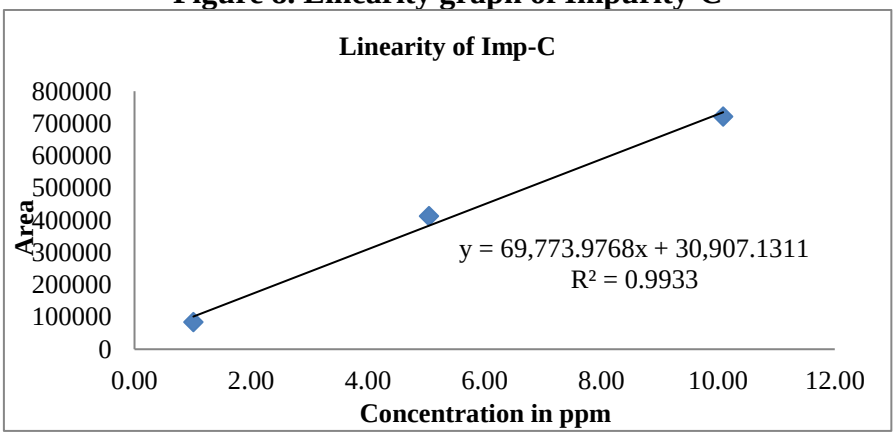
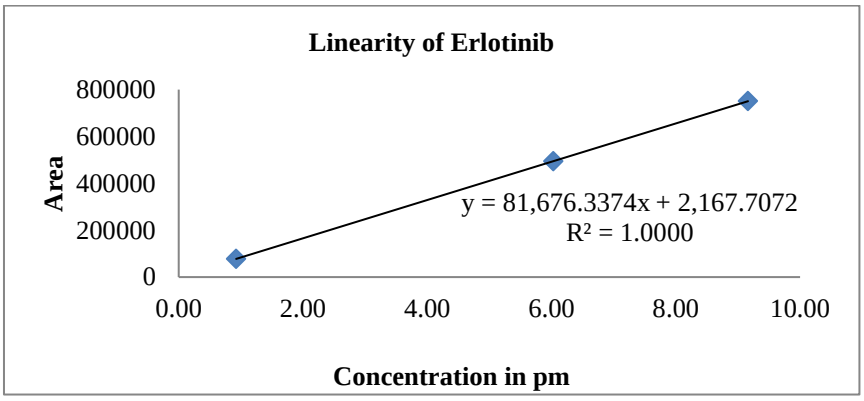


Table 8. Linearity for Erlotinib

S.No	Linearity Level	Concentration in ppm	Area response
1	0.1%	0.92	77793
2	0.5%	6.03	494111
3	1.0%	9.16	750699
Correlation coefficient (r)			1.0000
Square root of Correlation coefficient (r^2)			1.0000
Slope			81676.34
Intercept			2167.71
RRF			1.00

Figure 9. Linearity graph of Erlotinib



The linearity results for Erlotinib and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

4.5 Accuracy

Recovery of Erlotinib impurities in Erlotinib was performed. The sample was taken and varying amounts of Erlotinib impurities representing LOQ to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table 9.**

Table 9. Accuracy study of Erlotinib

S.No.	Theoretical (%)	% Mean Recovery		
		Impurity-A	Impurity-B	Impurity-C
1	LOQ	119.9	100	100
2	100	109	95.5	99.5
3	150	113.9	93.1	99.4

4.6 Solution stability of analytical solutions:

Standard and sample and spiked sample solutions were kept for 24 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of "old" prepared standard solutions with freshly prepared standard solutions.

Table 10. Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
12hrs	0.98	0.99
24hrs	0.98	0.99

Table 11. Results for solution stability of test solution at room temperature

Component	Initial Study	After 24Hrs	% Difference
Impurity-A	ND	ND	NA
Impurity-B	ND	ND	NA
Impurity-C	ND	ND	NA
Maximum unknown impurity	0.036	0.036	0.00
Total impurities	0.17	0.17	0.00

Table 12. Results for solution stability of test solution at refrigerator

Component	Initial Study	After 24Hrs	% Difference
Impurity-A	ND	ND	NA
Impurity-B	ND	ND	NA
Impurity-C	ND	ND	NA
Maximum unknown impurity	0.036	0.036	0.00
Total impurities	0.17	0.17	0.00

Table13. Results for solution stability of spiked sample at room temperature

Component	Initial Study	After 24Hrs	% Difference
Impurity-A	0.2146	0.2159	0.0013
Impurity-B	0.2200	0.2245	0.0045
Impurity-C	0.2177	0.2174	0.0003
Maximum unknown impurity	0.036	0.036	0.00
Total impurities	0.17	0.17	0.00

Table 1.4 Results for solution stability of spiked sample at refrigerator

Component	Initial Study	After 24Hrs	% Difference
Impurity-A	0.2146	0.2145	0.0001
Impurity-B	0.2200	0.2214	0.0014
Impurity-C	0.2177	0.2174	0.0003
Maximum unknown impurity	0.036	0.036	0.00
Total impurities	0.17	0.17	0.00

5.0 Results & Discussion:

A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Kromasil C18, 250 x 4.6 mm, 5µm column and the mobile phase consists of two Channels A and B. Channel-A: pH 2.80 phosphate buffer and Channel-B: Acetonitrile. The flow rate is 1.2 ml/min. The column temperature was maintained at 50°C and sample temperature was maintained at 5°C, injection volume 10µL and wavelength fixed at 248nm UV-detection.. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of selectivity, accuracy, linearity, precision, and stability of solution.

For selectivity, the chromatograms were recorded for standard and sample solutions of Erlotinib and its related substances. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of related substances in Erlotinib. There is no interference of diluent and placebo at Erlotinib and impurities peaks. The elution order and the retention times of impurities and Erlotinib obtained from individual standard preparations and mixed standard preparations are comparable.

The limit of detection (LOD) and limit of quantitation (LOQ) for Erlotinib standard 0.12&0.04µg/mL, impurity-A 0.02&0.5 µg/mL, impurity-B 0.03&0.10 µg/mL and impurity-C 0.01 & 0.03 µg/mL respectively.

The linearity results for Erlotinib and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for Erlotinib and its impurities found to be 1.000, 0.9991, 0.9999 and 0.9933 respectively.

The accuracy studies were shown as %recovery for Erlotinib and its impurities at specification level. The limit of % recovered shown is in the range of 80 and 120% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

For precision studies six replicate injections were performed. %RSD was determined from the peak areas of Erlotinib and its impurities. The acceptance limit should be not more than 10, and the results were found to be within the acceptance limits.

6.0. Conclusions

The new HPLC method developed and validated for determination of Erlotinib pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also

determining lower concentration of drug in its solid dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control.

Acknowledgment

The authors are grateful to Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Andhra Pradesh, India, for providing the facilities to carry this research work.

Conflict of interests

The authors claim that there is no conflict of interest.

7.0 References

1. https://en.wikipedia.org/wiki/Erlotinib
2. Jonathan Dowell, John D. Minna and Peter Kirkpatrick, Nature Reviews Drug Discovery. 2005; 4:13.
3. Honeywell, R., Yarzadah, K., Giovannetti, E., Losekoot, N., Smit, E.F., Walraven, M., Lind, J.S., Tibaldi, C., Verheul, H.M., Peters, G.J., 2010. Simple and selective method for the determination of various tyrosine kinase inhibitors used in the clinical setting by liquid chromatography tandem mass spectrometry. J. Chromatogr. B 878 (15–16), 1059–1068.
4. Padmalatha, M., Kulsum, S., Rahul, C., Reddy, T.M., Vidyasagar, G. Development and validation of UV. Spectrophotometric method for the determination of Erlotinib in tablet formulation imperial. J. Med. Org. Chem. 2011; 1 (1): 26–30.
5. Padmalatha, M., Kulsum, S., Rahul, C., Reddy, T.M., Vidyasagar, G. Spectrophotometric method for the determination of Erlotinib in pure and pharmaceutical dosage form. Int. J. Pharm. Res. Dev. 2011; 3 (6):103–109.
6. Padmalatha, M., Vanitha Prakash, K., Kusum, Syeda, Vishnu kumar, R., Wilson, K., Vivekananda, V. Development and validation of high performance liquid chromatographic method for the determination of Erlotinib. J. Pharm. Res. 2011; 4 (3): 637–638.
7. Rasoulzadeh, F., Asgari, D., Naseri, A., Rashidi, M.R. Spectroscopic studies on the interaction between Erlotinib hydrochloride and bovine serum albumin. DARU 2010; 18 (3): 179.

8. Usha Rani, G., Chandrasekhar, B., Devanna, N., 2011. Extractive colorimetric method development and validation for Erlotinib in bulk and tablet dosage form. J. Allied Pharm. Sci. 2011:1(07):176–179.
9. ICH, Q2 (R1) Validation of Analytical Procedures: Text and Methodology. ICH Harmonized Tripartite Guideline, 2005.