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Research Article

Development and validation of a stability indicating HPLC method for the quantification of impurities in Erlotinib hydrochloride dosage forms

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ABSTRACT

This work is aimed to develop a stability indicating high performance liquid chromatographic method for the analysis of Erlotinib HCl related compounds in pharmaceutical dosage forms. Separation was achieved on waters X-terra RP18 (150x4.6mm, 3.5µm) column using a gradient method. Here in this work mobile phase A is 0.01 M NaH_2PO_4 buffer (pH 4.5) and mobile phase B contains a mixture of 0.01 Na H_2PO_4, (pH 4.5) buffer and methanol in the ratio 20:80 (%V/V), respectively. The flow rate is 1.0 mL/min and the detection wavelength is monitored at 245 nm. The method was validated for specificity, limit of quantification, limit of detection, linearity, accuracy, method precision, intermediate precision, robustness and stability. Retention times of Erlotinib, impurity A and impurity B are 17.137, 6.650 and 10.346 minutes respectively. Linearity was observed over the concentration ranges of LOQ level to 1.5µg/mL of each impurity with correlation coefficient of 0.999. The RSD values of recoveries of impurities were found as less than 10%. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and thermal degradation. Extensive degradation was found in acid medium and alkaline medium. Minimum degradation was found in thermal and oxidative conditions while there was no degradation found in photolytic condition.

Keywords: Degradants; Erlotinib hydrochloride; HPLC; Stability indicating method.

INTRODUCTION

Erlotinib hydrochloride is used for the treatment of non-small cell lung malignancy, pancreatic cancer and several other types of cancer (Cohen *et al*., 2005). It is a reversible tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR) [2]. Its IUPAC name is N-(3-ethynylphenyl)-6, 7-bis (2-methoxyethoxy) quinazolin-4-amine Hydrochloride (Fig. 1). Erlotinib hydrochloride is synthesised from 4 - Chloro-6,7-bis (2-methoxyethoxy)-quinazoline and 3 – ethynylaniline in DMF. These raw materials may be present in bulk drug as an impurity; but levels of these impurities should not be more than 0.1%. If presence of impurities exceeds 0.1% with respect to the main drug, that must be identified and quantitatively determined by suitable analytical method. The quality of erlotinib hydrochloride is influenced by these impurities, which may leads to unnecessary toxicological effects. So, monitoring of these related substances is very important for controlling the quality of erlotinib in the final product of API and formulations.

Literature survey reveals that several analytical methods are available for determination of Erlotinib hydrochloride through spectrophotometric methods (Padmalatha *et al*., 2011; Rajesh *et al.,* 2011), through HPLC (Karunakara *et al.,* 2012; Faivre *et al.,* 2012; Chakravarthy *et al.,* 2011; Xu *et al.,*2011; Paez *et al.,*2004; Nageswari *et al.,* 2011; Ravi Kumar *et al.,* 2013; Latha *et al.,* 2013; Soheila *et al.,* 2013; Geetha *et al.,*2012; Pujeri *et al.,* 2009; Naveen kumat *et al.,* 2012; Chahbouni *et al.,* 2009) in bulk drug and dosage form and through LC-MS studies (Satheeshmanikandan *et al.,* 2012; Rivory *et al.,* 2001; Raju *et al.,* 2013; Andrea *et al.,* 2007) in biological matrix. Karunakara et al. (2012) presented a paper for the quantification of process impurities in Erlotinib hydrochloride active pharmaceutical ingredient. But they did not perform any degradation studies. Pujeri et al. (2009) reported stability indicating assay method for the determination of Erlotinib active pharmaceutical ingredient. But they were not studied the quantification of impurities. The reported analytical methods are mostly concentrated on content determination of Erlotinib either in bulk or formulations. So far no validated stability indicating analytical method for the determination of Erlotinib hydrochloride in the presence of degraded product in bulk drug as well as dosage forms were reported. Hence attempt was made to develop a stability indicating HPLC method for the degraded substances in Erlo-

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tinib Hydrochloride. Keeping in view of susceptibility of Erlotinib under variety of condition, it was felt that a HPLC method of analysis that separates the drug from the degradation products which are formed under ICH suggested condition such as hydrolysis, oxidation, photolysis and thermal degradation would be remarkable interest. These studies serve to give information on drugs inherent stability and help in the validation of analytical method to be used in the stability studies. Therefore, the objective of current research work was to study the degradation of Erlotinib under different ICH stress condition (ICH, 1995) and to establish specific, accurate and reproducible stability indicating HPLC method. This paper deals with the forced degradation of Erlotinib hydrochloride under stress condition like acid hydrolysis, alkaline hydrolysis, oxidation, photolysis and thermal. It also deals with the validation of the developed method for the accurate quantification of degradation products.

MATERIALS & METHODS

Reagents and chemicals

Working standards of Erlotinib Hydrochloride and Impurities were supplied by AR Life Sciences, (Hyderabad, India). Erlotinib Hydrochloride tablets were purchased from local pharmacies. The HPLC-grade of acetonitrile, methanol, analytical grade of Sodium dihydrogen Phosphate NaH_2PO_4 (AR grade) and ortho phosphoric acid were supplied by Merck (Darmstadt, Germany). High pure water was used from Millipore Milli -Q Plus water purification system (Billerica, MA).

Instrumentation and Chromatographic Conditions

The HPLC system used for method was Waters 2695 separation module consisting of binary pump plus auto sampler, auto injector (SM4 E 07 SM 4094 A, Singapore), online degasser, column oven and 2996 photodiode array (PDA) detector. The output signal was monitored and processed using Empower software, waters corporation, Milford, USA (Database Version 6.10.01.00). A waters X-terra RP18 (150 x 4.6mm, 5µ) column was used for LC studies. The flow rate of mobile phase was 1.0 mL/min. The HPLC gradient program was set as: time (min) / % solution B: 0/40, 25/100, 28/100, 30/40 and 35/40. The column temperature was maintained at 40°C and the detection was monitored at a wavelength 245 nm. The injection volume was 10 µL.

Preparation of standard and impurity stock solutions

A stock solution of Erlotinib hydrochloride (0.5 mg/mL) was prepared by dissolving an appropriate amount of drug in Acetonitrile–water 70:30 (v/v). Standard solutions containing 1.0 µg/mL were prepared from this stock solution for determination of related substances. A mixed impurity stock solution (0.1 mg/mL) was also prepared by dissolving the appropriate amount of impurities in Acetonitrile–water 70:30 (v/v).

Preparation of sample solution

Five Erlotinib hydrochloride 150 mg tablets were weighed and transferred into a clean, dry mortar and ground in to powder. Tablets powder equivalent to 100 mg drug was dissolved in 200 mL of Acetonitrile–water 70:30 (v/v) to give a solution containing 500 µg/mL.

METHOD VALIDATION

Specificity

Specificity of the analytical method is determined by identifying the peak purity and retention times of Erlotinib and impurities. Specificity of the method also determined by forced degradation of Erlotinib.

Forced degradation study

Forced degradation of Erlotinib hydrochloride was performed to provide an indication of the stability-indicating properties and specificity of the method. The stress conditions used for the degradation study included light (condition), heat (60°C), acid hydrolysis (5 N HCl), basic hydrolysis (5 N NaOH), aqueous hydrolysis and oxidation (10% H_2O_2).

Limit of detection and quantification

By injecting a series of solutions of known concentration, limit of detection (LOD) and limit of quantification (LOQ) for Erlotinib hydrochloride and for the two impurities were estimated at the amounts for which the signal-to-noise ratio was 3:1 and 10:1, respectively. Precision was also determined at LOQ level and % RSD was calculated for the peak area for each impurity and for Erlotinib.

Linearity and range

To test the response function of the method, solutions at six concentrations from LOQ to 150% of the analyte concentration (LOQ, 0.5, 0.75, 1.0, 1.25 and 1.5µg/mL) were prepared from the stock solution.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the value found. The accuracy of the concentrations for impurities, recovery was determined in triplicate for LOQ%, 0.1%, 0.2% and 0.3% of the analyte concentration (500 µg/mL) on drug product and recovery of the impurities was calculated.

Precision

The repeatability of the method was checked by six-fold analysis of 500 µg/mL Erlotinib hydrochloride spiked with 0.20% of each of the two impurities and the results are presented in Table 1.

Robustness

To determine the robustness of the method, the experimental conditions were deliberately changed. The

resolution of Erlotinib hydrochloride and the two impurities were evaluated. The mobile phase flow rate was 1.0mL/min; to study the effect of flow rate on resolution it was changed to 0.8 and 1.2 mL/min. The effect of pH was studied at pH 4.3 and 4.7 (instead of pH 4.5). The effect of column temperature was studied at 35°C and 45°C (instead of 40°C).

RESULTS AND DISCUSSION

Method development and optimization

The main objective of the chromatographic method was to separate critical impurities Impurity 1 and Impurity 2 and to elute Erlotinib hydrochloride as a symmetrical peak. Attempts were made using different C18 (Inertsil ODS-3, 150 mm X 4.6 mm, 5 µm particles and Waters Xterra RP_{18} 50 mm X 4.6 mm, containing 5 µm particles) HPLC columns, using different buffer pH (7.0) condition and using isocratic mobile phase elution. But at all above conditions, separation of impurities and degradation products was not satisfactory (Table 2). But the separation was satisfactory in presented adopted chromatographic conditions only. There was no interference of excipients with impurities peaks and Erlotinib hydrochloride peak. System suitability parameters were evaluated for Erlotinib hydrochloride and its two impurities. Tailing factor for all two impurities and Erlotinib hydrochloride was found less than 1.3. Resolution of Erlotinib hydrochloride and two potential impurities was greater than 2.0 for all pairs of compounds.

Specificity and stress studies

The Erlotinib hydrochloride and its known impurities (Impurity-A and Impurity-B) are eluted at different retention times (6, 10, 17 min respectively) and no blank peaks and placebo peaks are observed at the retention times of analytes (Fig. 2 and Fig. 3). The impurities are well resolved from the Erlotinib hydrochloride peak and the peak purity of known impurities and analyte peak purity passed. The purity of peaks obtained from stress samples was checked by use of the PDA detector. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrated the analyte peak homogeneity. Assay of stressed samples was performed by comparison with reference standards and the mass balance (% assay + % impurities + % degradation products) for stressed samples was calculated on peak area and included in Table 3. Chromatograms of degradation studies are presented in Fig. 4 to Fig. 8, which shows that more degradation was observed in under basic conditions (9.5%) and peak purity of impurities and analyte peaks are within the limits.

Limit of detection and quantification

The limit of quantification for Erlotinib, Impurity-A and Impurity-B were 0.02%, 0.03%, and 0.02% respectively. RSD (%) for precision at the LOQ concentration for Erlotinib, Impurity-A and Impurity-B were, 0.8%, 2.3% and 1.3%; respectively. Chromatograms of LOD and LOQ samples are presented in Fig. 9 and Fig. 10.

Linearity and relative response factors

Least-squares linear regression analysis was performed on peak areas and heights data. Solutions for testing linearity for the related substances were prepared by diluting the impurity stock solution to six different concentrations from the LOQ to 150% of the permitted maximum level of the impurity (i.e., the LOQ and 0.1%, 0.15%, 0.20%,0.25 and 0.30% for an analyte concentration of 500 µg/mL). The correlation coefficients, slopes, and *y*-intercepts of the peak areas and heights were reported. Results are summarized Table 4.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the value found. The accuracy of the concentrations for impurities, recovery was determined in triplicate for LOQ%, 0.1%, 0.2% and 0.3% of the analyte concentration (500 µg/mL) on drug product and recovery of the impurities was calculated. Results are summarised Table 5 which indicate that the method is highly accurate.

Precision and intermediate precision

The RSD (%) of peak area was calculated for each impurity. Inter-day and intra-day variation and analyst variation was studied to determine intermediate precision of the proposed method. Intra-day precision was determined by six-fold analysis of 500 µg/mL Erlotinib hydrochloride spiked with 0.20% of each of the two impurities. The % RSD of peak area for the two impurities namely Impurity-A and Impurity-B, in the study of the repeatability was 1.5% and 0.9%, respectively (Table 1). RSD (%) results of Erlotinib hydrochloride and its impurities for intermediate precision (intra and inter day repeatability) confirmed the method was highly precise.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of its reliability during normal usage. Robustness of the method is verified through variation of flow (± 0.1 mL/min), temperature (± 5°C) and buffer composition (± 2%). All system suitable results (Resolution NMT 2.0%) are within the limit.

Solution stability and mobile phase stability

The stability of Erlotinib hydrochloride and its impurities in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h during which they were assayed at 24 h intervals. Stability in the mobile phase was determined by analysis of freshly prepared sample solutions at 24 h intervals for 48 h and compared the results with those obtained

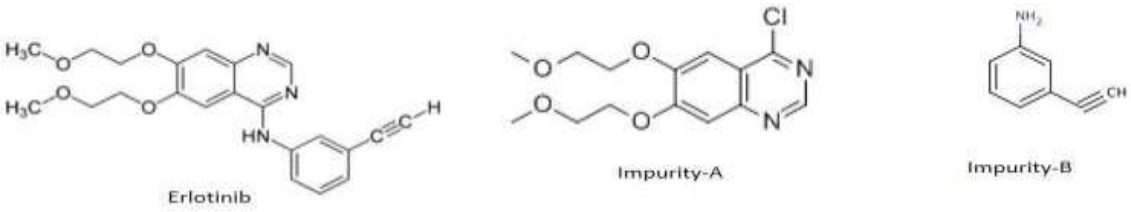

Figure 1: Chemical structure of Erlotinib and impurity A and Impurity

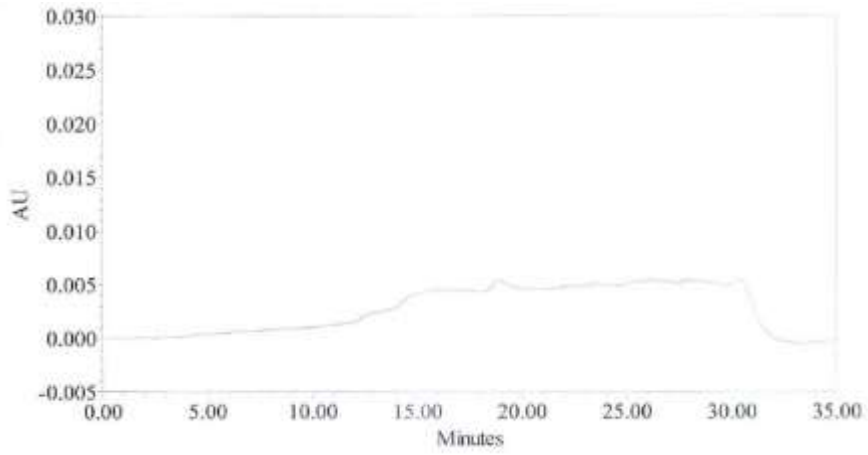

Figure 2: Typical chromatogram for Blank

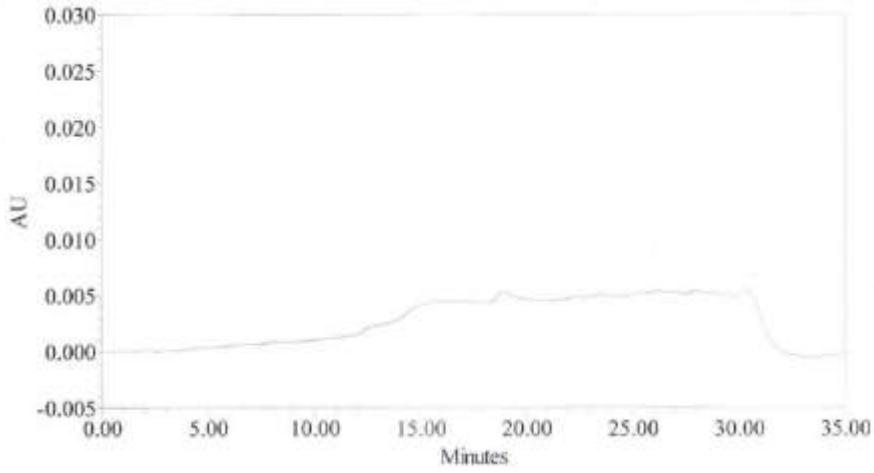

Figure 3: Typical chromatogram for Placebo

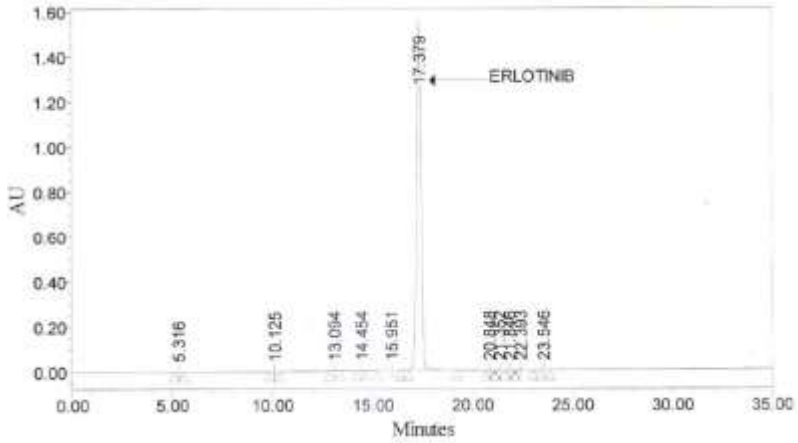

Figure 4: Typical chromatogram for acid sample

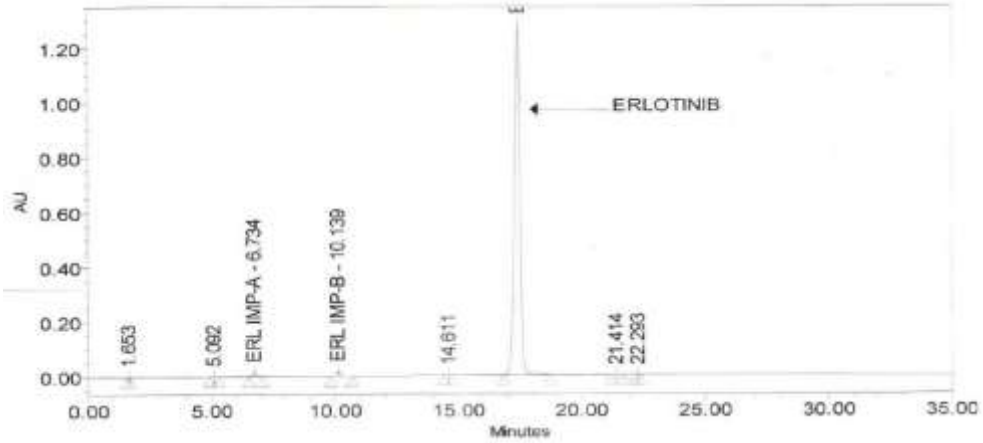

Figure 5: Typical chromatogram for base sample

Table 1: Summary of Results of Precision and Intermediate precision

Sample Prep No.	Precision		Intermediate Precision	
	Imp-A	Imp-B	Imp-A	Imp-B
1	0.21	0.20	0.22	0.19
2	0.19	0.19	0.19	0.21
3	0.22	0.22	0.21	0.20
4	0.19	0.21	0.18	0.19
5	0.22	0.21	0.21	0.20
6	0.21	0.18	0.20	0.18
Average	0.21	0.20	0.20	0.20
% RSD	8.0	7.3	6.8	6.4

Table 2: Results of different trials

Trail no.	HPLC conditions	Remarks
1	Column: Inertsil ODS-3V, 150 mm × 4.6 mm, 5 µm. Mobile phase: 0.01 M KH_2PO_4 buffer, and methanol (30:70, v/v/v)	Impurity-A and B co eluted.
2	Column: Inertsil ODS-3V, 150 mm × 4.6 mm, 5 µm Mobile Phase: 0.01 M Ammonium acetate buffer and methanol.	Impurity-B and blank peaks co eluted
3	Column: Waters X terra RP 18 150 mm × 4.6 mm, 5 µm Mobile Phase: 0.01 M Ammonium acetate buffer and methanol.	Impurity-B and blank peaks co eluted.
4	Column: Waters X terra RP 18 150 mm × 4.6 mm, 3.5 µm Mobile Phase-A: 0.01 M $NaH_2PO_4.2H_2O$buffer (pH 4.5) and Mobile Phase-B Methanol. Time/% B 0/40, 25/100,28/100,30/40,35/40.	Impurities and main peak are well separated.

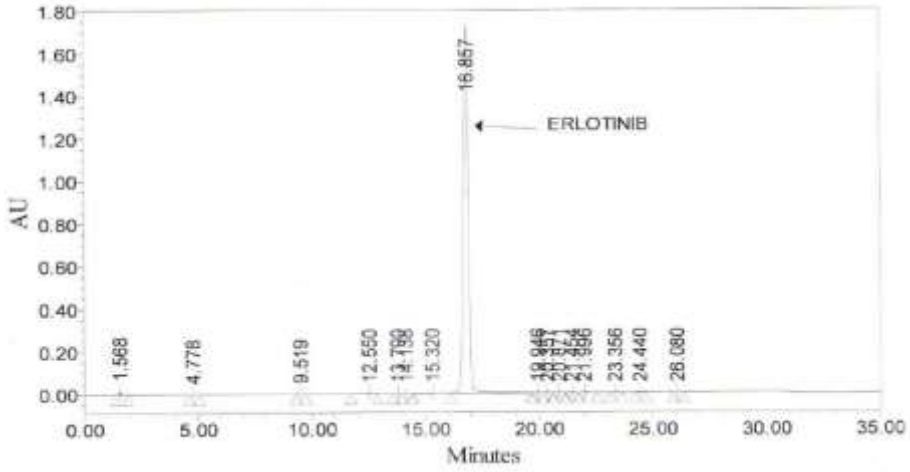

Figure 6: Typical chromatogram for thermal sample

Table 3: Summary of results of forced degradation studies

Sample details	% of degradation	% of Assay	Mass balance	Peak Purity
As such sample	0.15	100.6	-	Pass
Photolytic sample(1.2 million lux hrs)	0.43	99.5	99.2	Pass
Thermal sample(at 60°C for 7 days)	0.52	100.2	100.0	Pass
Base degradation(5N NaoH Solution)	9.50	89.5	98.3	Pass
Acid degradation(5N HCl Solution)	2.18	97.7	99.1	Pass
Peroxide degradation(10% H2O2)	1.80	97.4	98.5	Pass

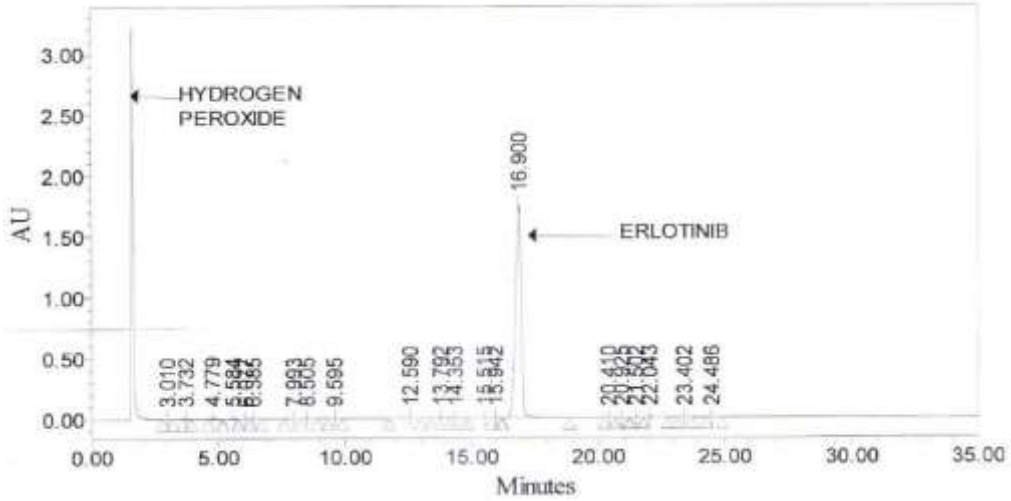

Figure 7: Typical chromatogram for peroxide sample

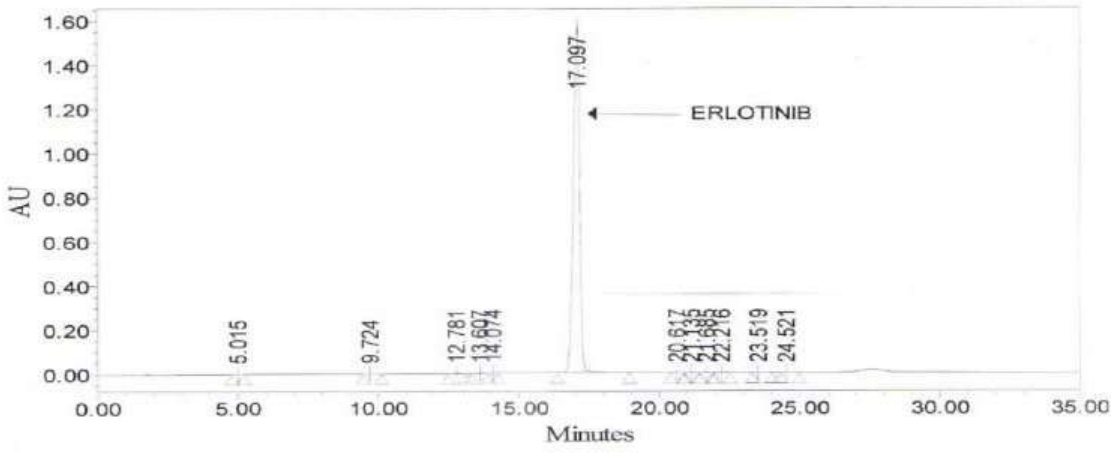

Figure 8: Typical chromatogram for photolytic sample

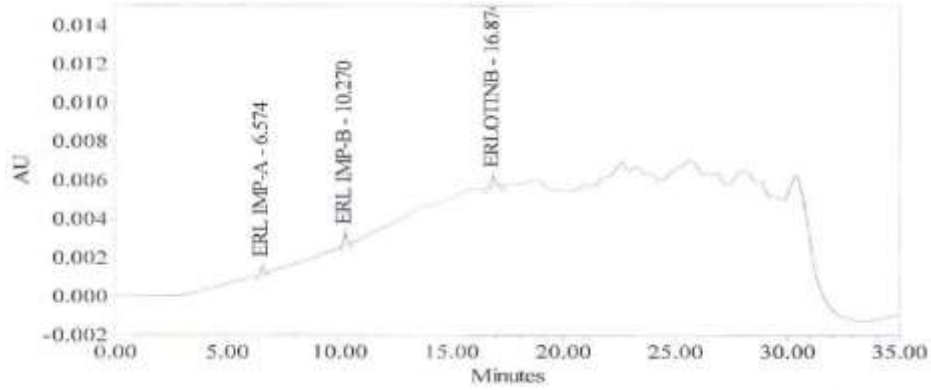

Figure 9: Typical chromatogram for LOD spiked solution

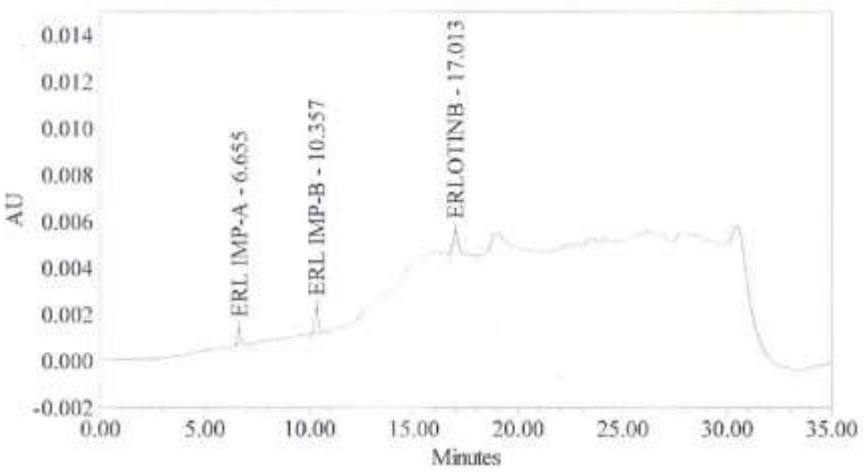

Figure 10: Typical chromatogram for LOQ spiked solution

Table 4: Linearity and RRF results

Name	Correlation Coefficient	Y bias at 100% level	RRF
Imp-A	0.999	-0.43	0.67
Imp-B	0.999	0.05	1.21
Erlotinib	0.999	0.12	1.00

Table 5: Accuracy results of Erlotinib hydrochloride and its impurities

Name	LOQ Level	50% level	100% level	150% level
Imp-A	97.4	99.0	96.6	94.0
Imp-B	98.2	97.4	95.9	94.8
Erlotinib	95.5	101.8	100.5	98.9

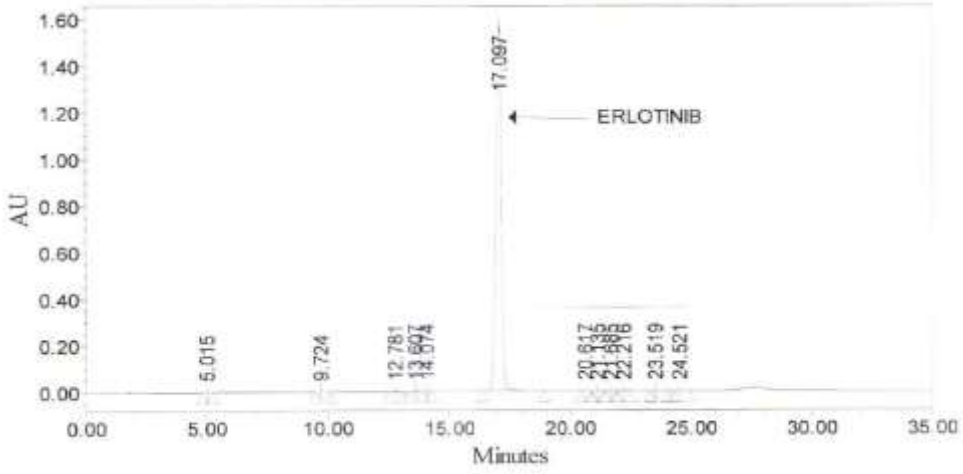

Figure 11: Typical chromatogram for sample

from freshly prepared reference standard solutions. The RSD (%) of the standard replicate and % of impurities was calculated for both the mobile phase and solution-stability experiments. No significant variations observed up to 48 hours at room temperature.

Method application

This method is applied to real Erlotinib tablets sample and chromatograms are shown in Fig. 11, which indicates that that all known and unknown impurities are found within 0.05% in Erlotinib tablets sample.

CONCLUSION

The reproducible gradient RP-HPLC method was developed for the analysis of Erlotinib hydrochloride and its related substances in pharmaceutical dosage forms. The method is precise, accurate, linear, robust, rugged, specific and with short runtime. Satisfactory results were obtained from validation of the method. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of samples of Erlotinib hydrochloride.

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