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Development And Validation Of Liquid Chromatographic Method For Estimation Of Erlotinib In Lipid Based Nanoformulation

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Abstract

Erlotinib (ERL) is an anti-cancer drug used to treat a variety of cancer. Using the HPLC method of analysis, the study aimed to quantify the drug in lipid based dosage form. Using isocratic system, samples were eluted, having eluent phase composition of phosphate buffer pH 4.5 and acetonitrile (40:60% v/v), in Orosil C-18 column at temperature 25 °C with a flow rate of 1 mL/min for run time of 12 min. The wavelengths of detection were set at 333 nm. A sharp and resolved peak of ERL at a retention time of 6.2 min was observed by the developed method. The limits of quantification and detection of the developed method were found to be 1.452 µg/mL^{-1} and 0.248 µg/mL^{-1}. The calibration curve's observed linearity range was between 2 and 50 µg/mL^{-1}, with an r^2 of 0.999. The developed and optimized method was validated in compliance with the ICH guidelines. The results of all validation parameters were within the acceptable range. The quantity of ERL in lipid based nanoformulation was determined using a simple and robust method and validated. The presence of excipients did not indicate any interference on the determination of ERL, showing method specificity.

Keywords: Erlotinib, RP-HPLC, Validation, Lipid based nanoformulation, Analytical method

1. Introduction

Erlotinib, N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine (Fig.1), represents a novel medication used in lung cancer therapy. Its mechanism of action involves inhibiting the epidermal growth factor receptor (EGFR). Specifically, it targets the tyrosine kinase activity of EGFR, a protein that is often overexpressed or mutated in various cancer types. Erlotinib binds reversibly to the adenosine triphosphate

(ATP) binding site on the receptor. To transmit signals, EGFR proteins must form homo dimers, requiring ATP to phosphorylate each other. This phosphorylation triggers a structural change within the cell, exposing sites for binding proteins that initiate a signaling cascade leading to the nucleus. By blocking ATP binding, erlotinib prevents autophosphorylation, halting the signaling process.

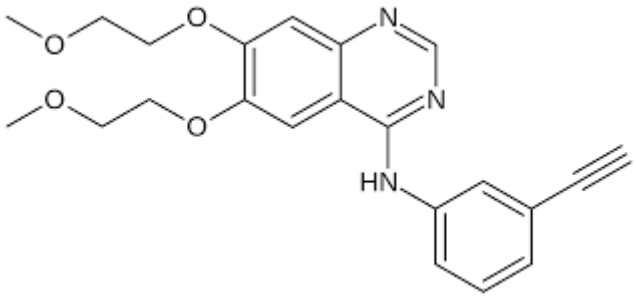

Fig.1 Structure of Erlotinib

Several analytical techniques have been documented for quantitatively assessing Erlotinib in pharmaceutical formulations. These include LC-MS, as highlighted by Honeywell et al. in 2010, presenting a straightforward and specific approach for determining various tyrosine kinase inhibitors utilized in clinical practice. Chahbouni developed and validated an LC-MS/MS method for quantifying Erlotinib, gefitinib, and imatinib in human plasma. Liquid-liquid extraction with imatinib as an internal standard was employed for sample pre-treatment. Additionally, Padmalatha et al. (2011) have introduced a UV spectrophotometric method for Erlotinib quantification in tablet formulations, and a separate spectroscopic method for both pure Erlotinib and pharmaceutical dosage forms. Furthermore, Padmalatha et al. (2011) have developed and validated a high-performance liquid chromatographic method for Erlotinib determination. Rasoulzadeh et al. (2010) conducted spectroscopic studies focusing on the interaction between Erlotinib hydrochloride and bovine serum albumin. Another method, presented by Usha Rani et al. in 2011, involves the development and validation of an extractive colorimetric technique for Erlotinib analysis in bulk and tablet dosage forms.

Few analytical approaches were utilized for the detection of erlotinib in plasma, bulk, and pharmaceutical dosage forms using liquid chromatography and liquid chromatography mass spectrometry (LC-MS) techniques, according to a thorough review of the literature. But there is no method available to quantify Erlotinib from lipid based nanoformulations. The aim of the current work is to develop a simple, precise, accurate, robust and stability indicating HPLC method for the determination of Erlotinib hydrochloride in lipid based dosage form followed by method validation as per current International conference on harmonization (ICH) guidelines.

2. Experimental work

2.1 Materials

ERL was received from Neon Labs, India as a kind gift sample. Phosphoric acid was procured from SD Fine Chemicals, Mumbai, India. Acetonitrile and rest reagents and chemicals used in method development were purchased from Merck, India.

2.2 Instrumentation and Chromatographic conditions

High-performance liquid chromatographic system (Jasco) equipped with Chromnav software was employed throughout the process. C_{18} column (dimensions, 250 mm length and 4.6 mm width, with 5.0 µm silica particles, Orosil) employed for chromatography. As per the initial screening and literature reports best possible and suitable solvents, i.e. acetonitrile and phosphate buffer (pH 4.5) were employed as mobile phase for isocratic elution, in 60:40 v/v ratio, with PDA detection at 333 nm.

2.3 Lipid-based nanoformulation

Lipid-based nanoformulation was prepared by hot melt emulsification/ultrasonication technique followed by high pressure homogenization. Solid lipids stearyl amine and Tripalmitn used for nanoparticulate formulation were kindly gifted by Gattefosse, India. Surfactant tween 80 was gifted by BASF, India. The prepared nanoparticulate formulation is labeled to contain 10 mg Erlotinib per 10 ml.

2.4 Methods

2.4.1 Method development

The drug ERL was separated and identified using an Orosil C-18 column at 25°C, with a mobile phase flow rate of 1 mL/min during a 8 minute run time, following customization of chromatographic conditions. An injection volume of 20 µL was utilized with a mobile phase composition mixture of phosphate buffer pH 4.5 (A) and acetonitrile (B) (40:60 percent v/v) under isocratic conditions. Elutes were detected at 333 nm.

2.4.2 Stock and working Standard Preparations

1 mg/mL strength stock solution of the drug (ERL) was prepared in methanol and stored at 4 °C. A final dilution of 100 µg/mL was prepared by mixing 1 mL of (1000 µg/mL) solution with 9 mL of mobile phase solvent (A: B 60:40 v/v). Three levels of samples were prepared for precision (a) 50 µg/mL, (b) 1 µg/mL, and (c) 0.2 µg/mL. For subsequent analysis, all of the solutions were kept at 20 °C.

2.5 Method Validation

All validation parameters, including system appropriateness, linearity, LOD, LOQ, accuracy, precision, robustness, and solution stability, were determined in accordance with ICH Q2 (R1) criteria

2.5.1 System Suitability Test

System suitability tests were conducted to assess the performance of the equipment in accordance with USP 24/NF 19 standards, ensuring reproducible outcomes. Prior to analyzing the sample batch, chromatographic system reproducibility was verified. The consistency of results across six batches (n = 6) of samples was

assessed, and the percentage-relative standard deviation (%RSD) was determined based on three factors: tailing factor, retention times, and theoretical plate.

2.5.2 Linearity

Linearity of the calibration curve was performed (n = 6) on the sample of the standard drug ERL of six dilutions in the range of 0.2–50 µg/mL. It was ensured by the detector's response of individual concentrations, and determined the r^2 value using the regression equation. A calibration curve was plotted between the average peak area and concentration of a sample of the working standard solution (10 µg/mL).

2.5.3 Precision

The precision was determined in terms of interday and intraday on three concentartions; 0.2 µg/mL, 1 µg/mL, and

50 µg/mL of ERL with %RSD of less than 2% of each concentration level.

2.5.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is the ability of an analytical method to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte which can be quantitatively determined with acceptable precision and accuracy. The LOD and LOQ were determined by the following equation according to ICH guideline:

LOD = 3.3 × σ/s,

LOQ = 10 × σ/s,

σ—Standard deviation of blank response,

s—Slope of regression equation.

2.5.5 Robustness

The importance of establishing robustness of the developed method by the comparison of a series of system suitability parameters obtained by employing deliberate changes is to ensure the integrity of analytical procedure whenever it is used. In the present study, the assay concentration 10.0 □g/mL of ERL was used for the determination of the robustness of the method.The following parameters were considered for the robustness:

(i) effect of pH of buffer in mobile phase (±0.2),

(ii) effect of organic solvent in mobile phase (±2%),

(iii) effect of wavelength (±2 nm),

2.5.6 Accuracy

For the accuracy test, the traditional addition approach (percent recovery) was employed. The percent recovery and percent RSD for each concentration were determined using the pre-estimated sample solution loaded with an additional 50, 100, and 150 percent of the standard ERL solution.

2.5.7 Solution Stability

The solution stability was determined at 12 µg/mL at 25 °C for 14 days and 2–8 °C for 30 days. The % recovery and % RSD of the experiment were calculated.

2.6 Application of validated method for assay of Erlotinib lipid based nanoformulation

10 mg equivalent quantity of lyophilized lipid based formulation was weighed and transferred to 10 mL volumetric flask. 3 mL of methanol was added to the flask and sonicated for 10 min. The volume was made up to the mark by using methanol.The solution was filtered through 0.45 □m syringe filter. The filtrate was further diluted to get the concentration equivalent to 10.0 □g/mL in diluent. The resulting solution was analyzed by the proposed HPLC method.

3. Results and Discussion

3.1 Method Validation

3.1.1 System Suitability Test

The analysis of the samples batch showed a reproducible result in factor retention time, tailing factor, and theoretical plate. The value of standard deviation (SD) and percentage of relative standard deviation (% RSD) indicate that the reproducibility of the chromatographic system is good (Table 1). All the values of %RSD below 2% followed the acceptance criteria. Well-resolved peaks of the drug and internal standard were observed. (Figure 2)

Table-1: System suitability

Compound	**Rt (min.)**	**ErlotInib (Area) mV/s**	**USP Tailing***	**USP Plate count***	**%RSD***
Erlotinib	6.250	5893312	1.21	5069	0.58

***Number of standard Injections analyzed are six.**

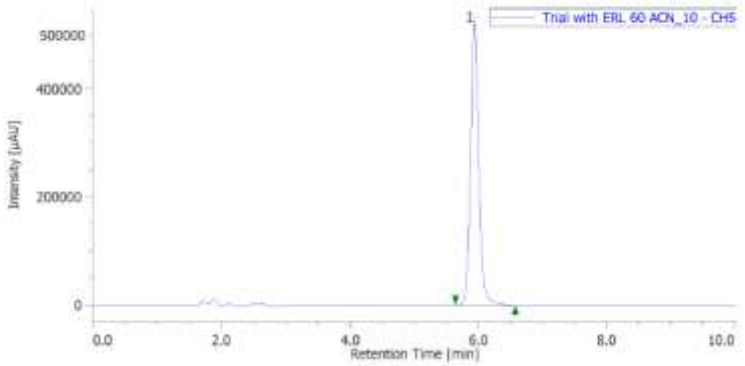

Figure 2- Chromatogram of Erlotinib and IS

3.1.2 Linearity

The calibration curve of the standard solution (2–20 µg/mL) was plotted in between average peak area and concentration. Results of the linear regression analysis of the calibration curves (n = 6) of ERL are depicted in Table 2. The r^2 value 0.999 and % RSD 1.23 indicated good linearity, with a limit of detection of 0.248 µg/mL.

Table 2 Linear regression analysis

Parameter	Erlotinib
Linearity range	2–20 µg/mL
Correlation Coefficient	0.9994
Regression Equation	y= 8.392x + 1278.5
Slope	8.392
Intercept	1278.5

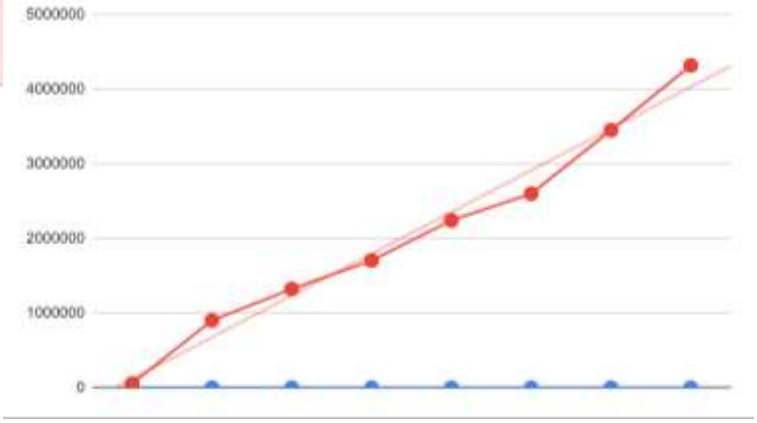

Figure 3- Calibration Curve of Erlotinib

3.1.3 Precision

Inter- and intra-day precision for three levels are presented in Table 3 and 4. The % RSD value of the precision was below 2%, which indicated that the developed method is precise.

Table 3 Intraday Precision

Sr. No	Conc.(µg/mL	Mean area	%RSD
1	0.2	65210	0.589
2	1	358210	0.125
3	50	641236	1.482

Table 4 Interday Precision

Sr. No	Conc.(µg/mL)	Mean area	%RSD
1	0.2	66253	1.192
2	1	360238	0.924
3	50	6512031	1.123

3.1.4 Quantification and Detection Limit.

The LOD and LOQ of ERL were found to be 1.452 µg/mL and 0.248 µg/mL, respectively.

3.1.5 Accuracy

The assay concentration for ERL was considered to be 10.0 □g/mL. The recovery was calculated at three levels of 50–150% of the assay concentration. The mean recovery for the ERL was 99.45 ± 0.16%. The recovery data for the ERL is shown in Table 5.

Table 5 Accuracy

Level	Mean Recovery
50	98.23
100	99.21
150	98.48

3.1.6. Robustness

Method robustness was checked for ERL estimation after employing the variables to the developed method.The data generated from the proposed method had significantly demonstrated that the HPLC method developed is robust. Results of the robustness parameter were tabulated in Table 6. It was observed that the% RSD for all the variable parameters was <1%, indicating the method is robust.

Table 6. Robustness

Parameter		% RSD of ERL
Mobile phase pH	4.8	0.95
	4.2	0.23
Mobile phase composition (ACN: Buffer)	72:38	0.97
	68:32	0.90
Wavelength in nm	335	0.54
	331	0.61

3.1.7 Solution Stability

The stability of the drug solution (ERL) was checked at a temperature of 25 °C for 14 days and 2–8 °C for 30 days for a concentration level of 12 µg/mL (MQC). The % RSD was found to be <2%. Results are shown in Table 7.

Table 7 Solution Stability

At 25 °C for 14 Days			At 2–8 °C for 30 Days		
Theoretical concentration	**Concentration found**	**%RSD**	**Theoretical concentration**	**Concentration found**	**%RSD**
12(µg/mL)	10.85(µg/mL)	1.25	12 (µg/mL)	11.85(µg/mL)	0.97

4. Application of the Method

The proposed method was utilized for the estimation of ERL in developed formulations.The lipid based nanoformulation assay was found in the range of 90–98% for different batches.

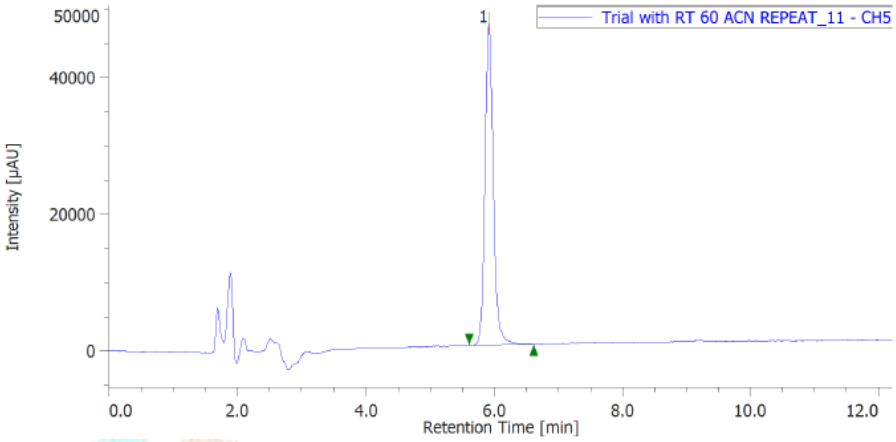

Fig.3 Chromatogram of ERL from lipid based nanoformulation

5. Conclusions

Erlotinib in pure and pharmaceutical formulation was identified and quantified using a simple to utilize, quick, validated, isocratic RP-HPLC technique with UV detection. The proposed method's selectivity, linearity, precision, and accuracy were confirmed by statistical data, and the method was successfully validated in accordance with ICH requirements. The method outlined can also be applied to the investigation of stability for analytical solutions. Given the significance of a brief retention period in drug analysis procedures, the present approach is suitable for use as a quality control instrument in Erlotinib assays within the pharmaceutical sector.

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