VALIDATED UV SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ANTICANCER DRUG ERLOTINIB HYDROCHLORIDE IN BULK AND TABLET FORMULATIONS[**]

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Two different simple, accurate, and precise UV spectrophotometric methods have been developed for the estimation of erlotinib hydrochloride in bulk and tablet forms by the zero-order (method I) and the zero-order AUC (method II). The drug was dissolved in a phosphate buffer solution (pH 7.4), and at 228.20 nm the zero-order (method I) and at 224.20–230.20 nm the zero-order AUC (method II) were developed. For both methods, erlotinib has linearity in the concentration range 10–70 μg/mL, with a correlation coefficient $R^2 > 0.99$. Both methods showed good reproducibility and recovery with %RSD less than 2 and an accuracy of 98.54–101.39 and 98.08–99.83%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.16, 3.51, and 21.41, 64.90 μg/mL, respectively, for both methods.

Keywords: *erlotinib hydrochloride, UV spectrometric, zero order, AUC, anticancer.*

УФ-СПЕКТРОФОТОМЕТРИЧЕСКИЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ ПРОТИВОРАКОВОГО ЛЕКАРСТВЕННОГО СРЕДСТВА ГИДРОХЛОРИДА ЭРЛОТИНИБА В НЕРАСФАСОВАННЫХ И ТАБЛЕТИРОВАННЫХ ФОРМАХ

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Разработаны два простых и точных УФ-спектрофотометрических метода определения гидрохлорида эрлотиниба в нерасфасованных и таблетированных лекарственных формах по спектру нулевого порядка (метод I) и по площади под кривой ошибок нулевого порядка (метод II). Препарат растворяли в фосфатном буферном растворе (pH 7.4) и проводили измерения методами I и II при 228.20 и 224.20–230.20 нм. Для эрлотиниба данными методами обнаружена линейная зависимость в диапазоне концентраций 10–70 мкг/мл с коэффициентом корреляции $R^2 > 0.99$. Методы показывают хорошую воспроизводимость и восстановление с RSD < 2% и точностью 98.54–101.39 и 98.08–99.83%. Пределы обнаружения (LOD) и количественного определения (LOQ) составляют 1.16, 3.51 и 21.41, 64.90 мкг/мл для обоих методов соответственно.

Ключевые слова: *гидрохлорид эрлотиниба, УФ-спектрометрия, спектр нулевого порядка, площадь под кривой ошибок нулевого порядка, противораковое средство.*

Introduction. Erlotinib hydrochloride is a small molecule with the chemical name *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine [1]. Erlotinib hydrochloride is used to treat lung cancer, pancreatic cancer, and several other types of cancer [2]. It is a tyrosine kinase inhibitor that acts on the epidermal growth factor receptor (EGFR) [3]. It binds reversibly to the adenosine triphosphate (ATP) binding site of the receptor. For the signal to be transmitted, two members of the EGFR family need to come together to form a homo dimer [4].

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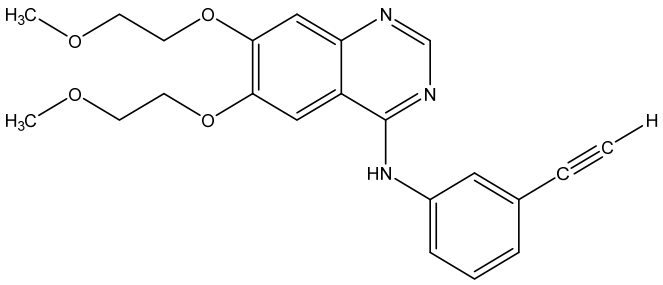
The literature survey reveals methods like high-performance liquid chromatography (HPLC) [2–8], simultaneous liquid chromatography (HPLC) [9], liquid chromatography-mass spectrometry (LC-MS/MS) [10, 11], simultaneous liquid chromatography-mass spectrometry (LC-MS/MS) [12, 13], hydrophilic interaction liquid chromatography and tandem mass spectrometry (HILIC-MS/MS) [14], and matrix-assisted laser desorption ionization (MALDI)-mass spectrometry (MS) [15] for the estimation of erlotinib hydrochloride individually and in combination with other drugs. No individual ultraviolet (UV) spectrophotometric method has been reported yet for the zero-order (method I) [16–18] and the zero-order AUC (method II) [19–21]. Hence, the current study deals with the development and validation of zero-order and AUC UV spectrometry absorbance methods in bulk and tablet dosage forms. The developed methods are further validated for precision, accuracy ruggedness, and sensitivity according to the ICH Q2R1 guidelines [22].

Methodology. *Chemicals and reagents.* All the chemicals and reagents were purchased from Qualigens Fine Chemicals, Mumbai, India. Erlotinib hydrochloride was received as a gift sample from MSN Laboratories Private Limited Unit-II, Telangana:



Instrumentation. Weighing Balance: Shimadzu AUX-120. Ultrasonicator: ENERTECH Electronics. Spectrophotometer: UV-2450 Shimadzu, Japan. Software: UV Probe 2.21. Sample cell: 1 cm quartz cuvette. Lamp: deuterium lamp wavelength range 200–400 nm. Detector: silicon photodiode, Photomultiplier R-928. Scan speed: medium. Spectral slit width: 1.0 nm.

Preparation of the pH 7.4 phosphate buffer solution. We accurately weighed and transferred about 3.40 g of potassium dihydrogen phosphate into 400 mL of purified water. To this solution 0.78 g of sodium hydroxide was added, and the pH of the solution was adjusted to 7.4 (±0.05) and further diluted to 500 mL with purified water.

Preparation of standard stock solutions. We accurately weighed 10 mg of erlotinib hydrochloride by the addition method [23]; 100 mL of the phosphate buffer solution was further added to obtain a concentration of 100 µg/mL and pH 7.4. The working standards were prepared by diluting the standard stock solution with the same solvent.

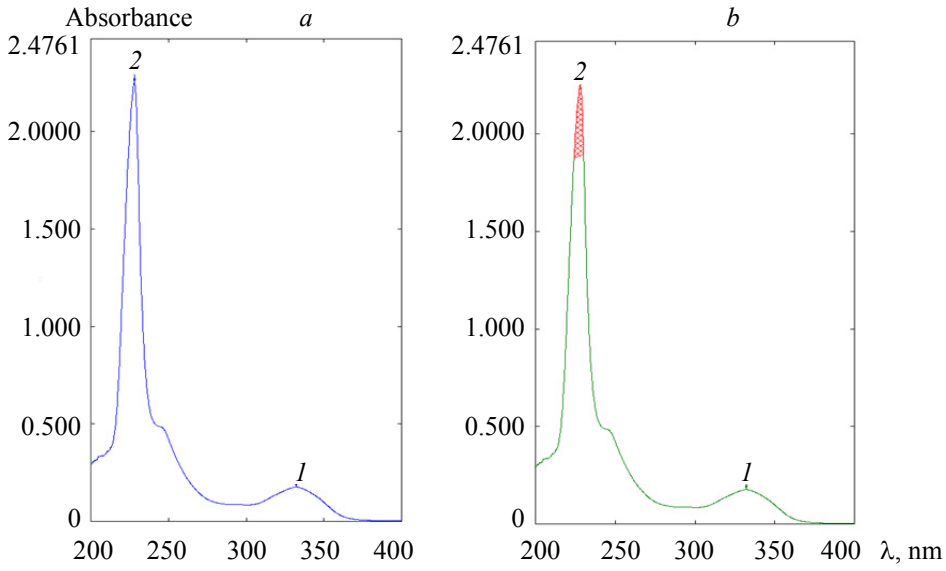


Fig. 1. UV-spectrum of erlotinib hydrochloride (a) method I and (b) method II.

Selection of the analytical wavelength. An appropriate 1 mL volume from the stock solution was transferred to a 10 mL volumetric flask. The volume was adjusted up to the mark with the same solvent to obtain a concentration of 10 µg/mL. The resultant solution was scanned in the UV range (200–400 nm) in a 1.0 cm cell against the solvent blank. The λ_{max} of erlotinib hydrochloride was found to be 228.20 nm (method I), and two wavelengths, 224.20 nm to 230.20 nm, were selected for determining the area under the curve (Fig. 1).

Linearity studies. For the linearity study, solutions of erlotinib hydrochloride of different concentrations (10, 20, 30, 40, 50, 60, and 70 µg/mL) were prepared using the standard stock solution, diluted with the phosphate buffer solution (pH 7.4). It was further analyzed by the proposed methods, and the obtained data were utilized to plot calibration curves as shown in Table 2 (methods I and II) (Fig. 2).

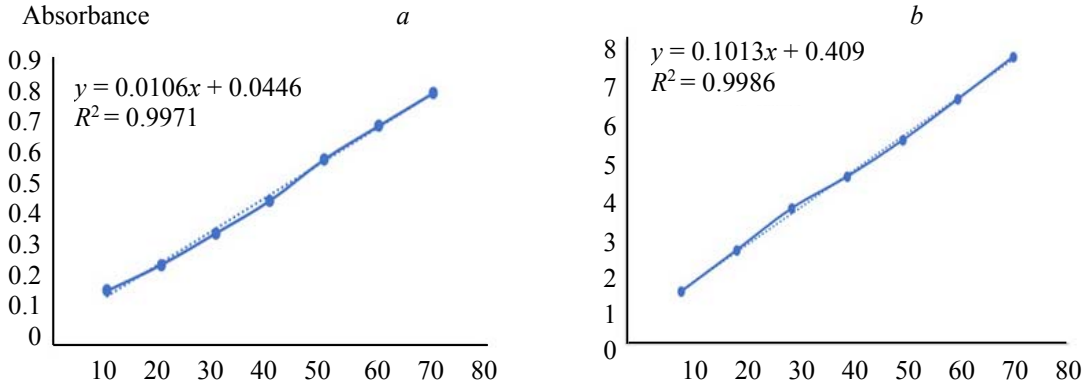


Fig. 2. Zero-order linearity of (a) method I and (b) method II.

Analysis of the tablet formulation. Twenty tablets were accurately weighed, and the average weight was determined. The powder drug equivalent to 10 mg of erlotinib hydrochloride was transferred into a 100 mL volumetric flask, and the volume was made up to the mark with the phosphate buffer solution (pH 7.4), sonicated for 15 min, and filtered through 0.45 µm filter paper. From this prepared solution, a 40 µg/mL concentration solution was prepared after appropriate dilution. The AUC was recorded in the 224.20–230.20 nm spectral region (method II), and the concentrations were determined using the respective linear regression equations. The analysis procedure was repeated for six times with the same concentration at 228.20 nm, as depicted in Table 1 (methods I and II).

TABLE 1. Analysis of the Marketed Formulations, Zero-Order (Method I) and Zero-Order AUC (Method II)

Method	Label claim, mg	Concentration, µg/ml	% Found (n = 6)	SD	% RSD
I	150	40	98.12	1.23	1.25
II	150	40	98.04	0.28	0.29

Validation. The method was validated in terms of accuracy, precision, ruggedness, sensitivity, and repeatability. The accuracy of these proposed methods was estimated by recovery studies. To the pre-analyzed sample solutions of the 30 µg/mL concentration, a known amount of the standard stock solutions was added at 80, 100, and 120% levels. The solutions were analyzed by the proposed methods. The experiments were performed three times at each level for each method. The recovery percentages 98.54–101.39% and 98.08–99.83% for method I and method II indicate that these methods are accurate with an acceptable error (Table 2).

The precision was determined as intra-day and inter-day variations. The intra-day precision was determined by analyzing 30, 40, and 50 µg/mL of the erlotinib hydrochloride solution three times in the same day. The inter-day precision was determined by analyzing 30, 40, and 50 µg/mL of the erlotinib hydrochloride drug solution daily for three consecutive days over a week (Table 2). The repeatability was determined by analyzing 40 µg/mL of erlotinib hydrochloride six times, and the results are reported in Table 2 for both methods. The ruggedness of the proposed method was determined by the analysis of aliquots from the homogeneous slot by two analysts using the same operational and environmental conditions, and the results are reported in Table 2 for both methods.

TABLE 2. Validation Parameter for Erlotinib

Parameter	Method I	Method II
Working wavelength, nm	228	228
Linearity range, μg/ml	10–70	10–70
Coefficient correlation [R^2]	0.997	0.998
Precision, %RSD		
Inter-day [n=3]	0.95–1.45	0.24–1.90
Intra-day [n=3]	0.75–1.60	0.80–1.63
Repeatability [n=6]	1.60	0.79
Ruggedness, %RSD		
Analyst I [n=6]	0.57	0.18
Analyst II [n=6]	0.83	0.52
% Recovery [n=3]		
Tablet	98.54–101.39	98.08–99.83
%RSD	0.66–1.36	0.29–1.02

The sensitivity of the proposed methods was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). It was calculated using the formulas LOD = 3.3N/S and LOQ = 10N/S, where N is the average standard deviation of the absorbance of the erlotinib drug (n = 6) taken as a measure of the noise, and S is the slope of the corresponding calibration curve. The LOD and LOQ were found to be 1.16, 3.51, and 21.41, 64.90 μg/mL, respectively, for both methods.

Result and discussion. In the pH 4.7 phosphate buffer solution, erlotinib hydrochloride showed maximum absorbance at 228.20 nm. In methods I and II, erlotinib hydrochloride followed linearity in the concentration range 10–70 μg/mL. The amounts of erlotinib hydrochloride estimated by methods I and II were found to be 98.12 and 98.04%, respectively, for the tablet formulation. The accuracy of the method was determined by calculating the mean percentage recovery. It was determined at 80, 100, and 120% levels. In both methods, the precision was studied as repeatability (%RSD < 2) and inter- and intra-day variations (%RSD < 2). The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The linearity, % recovery, precision, repeatability, and ruggedness data are presented in Table 2.

Conclusions. A method for the estimation of erlotinib hydrochloride in bulk and tablet dosage forms was developed. The drug shows absorption maxima at 228.20 nm. The spectrophotometric method linear response was obtained in the concentration range 10–70 μg/mL, with a correlation coefficient of 0.99. The method was statistically validated according to the ICH guidelines. The present research work is simple, economical, accurate, and precise and can be used for the routine analysis of erlotinib hydrochloride from its bulk and tablet formulations.

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