DEVELOPMENT AND VALIDATION OF THE ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ERLOTINIB HYDROCHLORIDE

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A simple, accurate, novel, safe, and precise method was developed for the estimation of erlotinib hydrochloride in tablet dosage form using a mixture of methanol and acetonitrile (50:50% V/V). The maximum and subsidiary peak absorption of erlotinib hydrochloride were noted at 247 and 333 nm, respectively. Erlotinib hydrochloride follows Beer's law in the concentration range of 5–30 μg/mL (r^2 = 0.9992). In the proposed method, the subsidiary peak absorption wavelength of 333 nm was used to estimate the concentration of erlotinib hydrochloride in tablets. The linear regression equation was found to be y = 0.0461x + 0.0164. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines, and the values of accuracy, precision, and other statistical variables were found to be in accordance with the prescribed values.

Keywords: *erlotinib, spectrophotometry, statistical variables, validation.*

РАЗРАБОТКА И ВАЛИДАЦИЯ УФ-СПЕКТРОФОТОМЕТРИЧЕСКОГО МЕТОДА ОПРЕДЕЛЕНИЯ ГИДРОХЛОРИДА ЭРЛОТИНИБА

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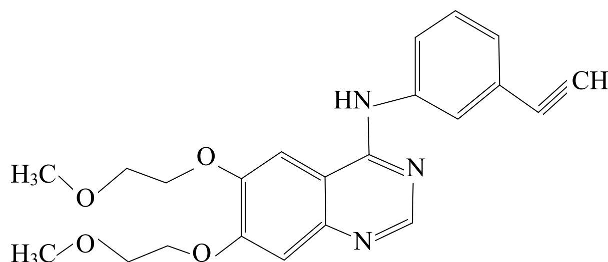
Разработан простой, точный и безопасный метод определения содержания эрлотиниба гидрохлорида в лекарственной форме в виде таблеток с использованием смеси метанола и ацетонитрила (50:50 об.%). Максимальное и дополнительное пиковое поглощение эрлотиниба гидрохлорида отмечено на длинах волн 247 и 333 нм, что подчиняется закону Бера в диапазоне концентраций 5–30 мкг/мл (r^2 = 0.9992). В предлагаемом методе для оценки концентрации эрлотиниба гидрохлорида в таблетках использована длина волны дополнительного пикового поглощения 333 нм. Уравнение линейной регрессии y = 0.0461x + 0.0164. Разработанный метод проверен в соответствии с рекомендациями Международной конференции по гармонизации (ICH). Показатели точности и другие статистические переменные соответствуют заданным значениям.

Ключевые слова: *эрлотиниба гидрохлорид, спектрофотометрия, статистические переменные, валидация.*

Introduction. The chemical name of erlotinib hydrochloride is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine:



The molecular weight and pK_α value of erlotinib hydrochloride are 429.90 and 5.42 at 25°C, respectively. Erlotinib hydrochloride is very slightly soluble in water, slightly soluble in methanol, and insoluble in acetonitrile, acetone, ethyl acetate, and hexane. With a decrease in pH, the aqueous solubility of erlotinib hydrochloride increases due to the protonation of the secondary amine (pH < 5) [1, 2].

Erlotinib hydrochloride has been used to treat several types of cancer including non-small-cell lung cancer and pancreatic cancer. It inhibits the tyrosine kinase receptor, which acts on the epidermal growth factor receptor. Erlotinib exhibited a survival benefit in lung cancer in phase III trials. The US Food and Drug Administration has approved the use of erlotinib for the treatment of certain types of lung cancer [2].

Various ultraviolet (UV) spectrophotometry methods have been recently developed. Among them, a wavelength of 247 nm was used to estimate data [2]. In a derivative spectroscopy method developed by Sereya et al., a wavelength of 247 nm was used to derive analytical data [3]. Mathrusri et al. used 0.1 N HCl aqueous solution, acetate buffer (pH 4.0), and phosphate buffer (pH 5.0) as diluents containing water in three methods, with wavelengths of 342.37, 342.40, and 341.08 nm, respectively [4]. In the presence of water during isolation and formulation, hydrolysis may occur and some functional groups may undergo hydrolysis. Generally, hydrolysis occurs in the presence of an acid or base but sometimes may occur in a neutral condition even in the presence of moisture [5]. In the method proposed here, no water was used to prevent hydrolysis.

Analytical techniques such as HPLC [6–8], LC-MS [9], UPLC [10], and HPTLC [11], have been used to determine the amount of erlotinib. UV spectrophotometry is a simple, time-saving, and cost-effective technique compared with HPLC, HPTLC, and LC-MS and those available in most pharma laboratories. Hereafter, erlotinib hydrochloride is referred to as Erl · HCl. The proposed method was as per the International Conference on Harmonization (ICH) guidelines [12].

Experimental. A pure sample of Erl · HCl was obtained as a gift sample from the manufacturer. Methanol (AR grade, LOBA chemicals Pvt. Ltd., India) and acetonitrile (AR grade, SD Fine Chemicals, Mumbai, India) were used to develop the method. Each tablet containing 25 mg of Erl · HCl and prepared by different manufacturers was procured from the local market. A LABINDIA UV/VIS 3000 plus spectrophotometer with 1-cm matched quartz cells was used for the estimation.

Babu et al. reported that in base hydrolysis, more than 30% degradation of the drug occurred after 24 h. Acid hydrolysis and neutral conditions resulted in 10 and 4% degradation, respectively. Less degradation was observed in the neutral medium in the method, which consisted of water [13]; thus, a neutral medium was chosen to prepare the solution of the drug.

The solubility of the drug was determined at 25°C. An excess amount of the drug (i.e., 25 mg) was added to 10 mL of volumetric flasks containing different solvents, namely methanol, isopropyl alcohol, and acetonitrile. However, the authors observed the presence of some particles in methanol. The drug was found to be insoluble in acetonitrile and isopropyl alcohol but to be completely soluble in a combination of methanol and acetonitrile (1:1 ratio).

A known amount of the drug was diluted in the methanol:acetonitrile mixture (1:1) and further diluted to obtain the solution with a concentration of 15 μg/mL. The solution was scanned under a UV wavelength of 200–400 nm. Although the maximum absorption was observed at 247 nm, a subsidiary peak was noted at 333 nm (Fig. 1). Latha et al. used HPLC for estimating the concentration of Erl · HCl and found the maximum absorption of the drug at 246.58 nm and a subsidiary peak at 333.02 nm in methanol, a finding that is in accordance with our results [6]. No change was noted in absorption at 333 nm until 24 h at 22°C in an air-conditioned room where direct sunlight was absent. However, when the solution was stored for more than

10 h at 30°C, the peak observed at 333 nm was diminished and a different peak was observed at 345–347 nm. Thus, we selected a wavelength of 333 nm for the analytical quantification of the drug.

Although UV absorption peaks observed at 247 and 345 nm have been used to estimate the drug concentration through different UV spectrometry techniques, this is the first study to use a wavelength of 333 nm for the analysis. No significant absorption was observed for the dilution mixture, indicating that it did not interfere in the absorption of the drug. Hence, the mixture of methanol: acetonitrile (1:1) was used as a diluent.

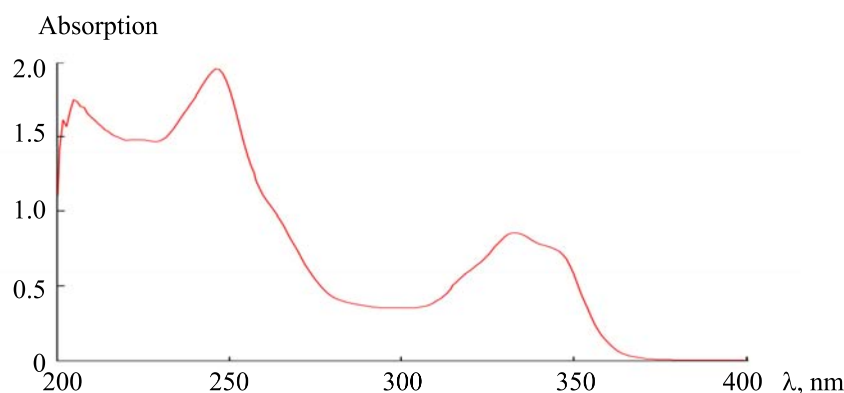


Fig. 1. UV absorption of API Erl · HCl.

Results and discussion. We accurately weighed 50 mg of the drug and transferred it into a 50-mL flask. Then, 40 mL of the diluent was added up to the mark, and the mixture was sonicated for 5 min to degas it. The concentration of the standard solution was 1,000 μg/mL.

We pipetted 25 mL of the solution from the standard solution (1,000 μg/mL), transferred it into 250 mL of the volumetric flask, added approximately 200 mL of the diluent, sonicated to degas it for 10 min, and further diluted it up to the mark. The solution concentration was 100 μg/mL and was used as a working standard solution.

Furthermore, different volumes were pipetted from the working standard solution, transferred into different volumetric flasks, and further diluted to prepared solutions of different concentrations ranging from 5 to 30 μg/mL. The calibration curve was plotted as the concentration against absorbance. The absorption values of different dilutions are listed in Table 1, and an overlay spectrum is shown in Fig. 2. The regression equation was found to be $y = 0.0461x + 0.0164$ (Fig. 3); other data: wavelength 333 nm, Beer's law limit 5–30 μg/mL, correlation coefficient $r^2 = 0.9992$, slope $m = 0.0461$, intercept $c = 0.0164$, LOD = 0.97 μg/mL, LOQ = 2.95 μg/mL.

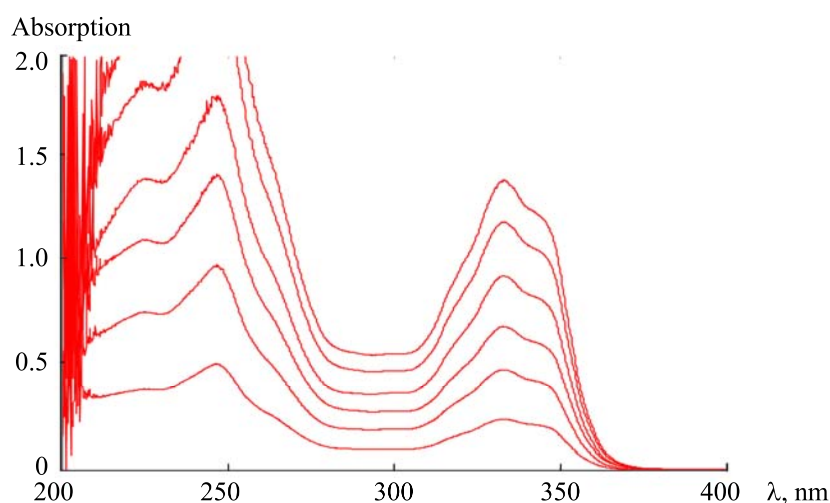


Fig. 2. Overlay spectrum for 5–30 μg/mL.

To obtain precision data, five replicants of the solution with a concentration of 20 μg/mL were analyzed absorbance A = 0.9262, 0.9289, 0.9301, 0.9293, 0.9274; mean 0.9284; SD = 0.001564, and the value of %RSD = 0.1685 was calculated. The accuracy (recovery) data were obtained by the addition of 50, 100, and 150% of the preanalyzed solutions to a fixed standard API weight. The precision and recovery data are listed in Table 2.

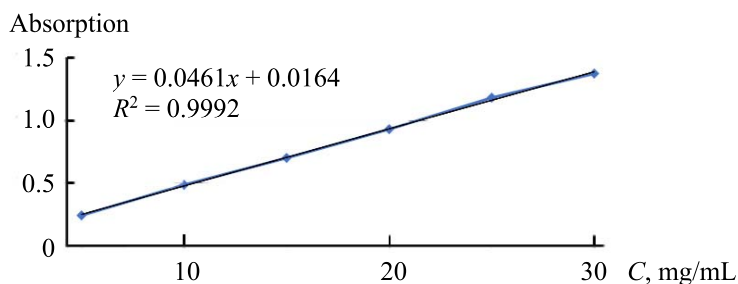


Fig. 3. Linearity graph of API Erl · HCl.

TABLE 1. Absorption of Different Concentrations for Linearity

C, μg/mL	Absorption (n = 3)
5	0.2449
10	0.4826
15	0.7001
20	0.9275
25	1.1894
30	1.3847

TABLE 2. Accuracy (Recovery Data)

Amount added (level) (added 1,007.6 μg/mL preanalyzed) and made vol. 100 mL	Mean absorbance, n = 3 (take 10 mL and dilute it up to 100 mL)	Amount recovered, mg	Amount recovered, %
5 mL (50%)	0.3840	8.0043	99.58
10 mL (100%)	0.6158	13.0435	99.75
15 mL (150%)	0.8479	18.0891	99.72

N o t e. Amount taken (standard) 3 mg.

Ten tablets containing Erl · HCl (25 mg each) were weighed and ground to a fine powder. An amount equal to 25 mg was taken in a 20-mL volumetric flask, and 20 mL of the diluent was added. The flask was sonicated for approximately 10 min to solubilize the drug present in the tablet form, and the resulting solution was filtered through a Whatman filter paper No. 1 into another 25-mL volumetric flask and diluted up to the mark with the diluent (1,000 μg/mL). Furthermore, we pipetted 1 mL of the prepared solution and transferred it into a 50-mL volumetric flask, also diluting it up to the mark. This solution was used to examine the UV absorption. The related results are summarized in Table 3.

The limit of detection (LOD) and limit of quantification (LOQ) of Erl · HCl were determined using the standard deviation of the response and slope approach using the following equations: LOD = 3.3×SD/Slope, LOQ = 10×SD/Slope, where SD is the standard deviation, and LOD and LOQ of Erl · HCl were found to be 0.97 and 2.95 μg/mL, respectively.

TABLE 3. Assay Quantification

Commercial formulation (tablets)	Labeled amount, mg	Amount observed, mg, n = 3	Amount observed, %
Tablet 1	25	24.85	99.4
Tablet 2	25	25.19	100.7

The method proposed here was found to be adequately rugged. To examine ruggedness, the assay estimation was performed by another analyst using the same instrument. The following ruggedness data were obtained: for commercial formulation tablet 2 with labeled amount 25 mg average absorption (equal to 20 μg/mL, $n = 3$) $A = 0.9402$, SD = 0.0027, %RSD = 0.287, amount observed is 25.05 mg (100.2%)

Conclusions. The solvents used in this study did not interfere with the estimation. Hence, the UV spectrophotometric method was found to be simple, accurate, economical, and rapid for the estimation of Erl · HCl in bulk and tablet dosage forms. The proposed method can be successfully applied for the routine analysis of Erl · HCl-containing dosage forms.

REFERENCES

1. http://www.accessdata.fda.gov/drugsatfda_docs/la bel/2008/021743s010lbl.pdf.
2. V. Ashok Chakravarthy, B. B. V. Sailaja, *Europ. J. Pharm. Med. Res*., **3**, No. 12, 609–624 (2016).
3. K. Sereya, S. T. Latha, D. Kamalakannan, M. Jambulingam, M. Anilkumar, S. Ananda Thangadurai, *Int. J. Pharm. Health Care Res*., ISSN: 2306-6091, **1**, No. 1, 23–27 (2013), www.ijphr.com.
4. M. Mathrusri, Annapurna, B. Venkatesh, R. Krishna Chaitanya, *Chem. Sci. Transact*., **3**, No. 2, 840–846 (2014), doi: 10.7598/cst2014.828.
5. Camille Wermuth, David Aldous, Pierre Raboisson, Didier Rognan, *The Practice of Medicinal Chemistry*, 4th ed. (2015), doi: 10.1016/C2012-0-03066-9.
6. S. T. Latha, S. AnandaThangadurai, M. Jambulingam, K. Sereya, D. Kamalakannan, M. Anilkumar. *Arab. J. Chem*., **10**, No. 1, S1138–S1144 (2017), doi: 10.1016/j.arabjc.2013.02.006.
7. Soheila Bolandnazar, Adeleh Divsalar, Hadi Valizadeh, Arash Khodaei, Parvin Zakeri-Milani, *Adv. Pharm. Bull*., **3**, No. 2, 289–293 (2013), doi: 10.5681/apb.2013.047.
8. V. S. Saravanan, B. Mallikarjuna Rao, *J. Drug Delivery Therap*., **3**, No. 1, 50–54 (2013).
9. Anna Svedberg, Henrik Green, Anders Vikström, Joakim Lundeberg, Svante Vikingsson, *J. Pharm. Biomed. Anal.*, **107**, 186–195 (2015), doi: 10.1016/j.jpba.2014.12.022.
10. Takahiro Suga, Miki Shimada, Masamitsu Maekawa , Hiroyuki Suzuki, Masaru Mori, Tatsuma Okazaki, Akira Inoue, Hiroaki Yamaguchi, Nariyasu Mano, *Chromatography*, **38**, 95–100 (2017), doi: 10.15583/jpchrom.2017.007.
11. V. Rajesh, B. Anupama, V. Jagathi, K. Varaprasad, *Int. J. Biol. Med. Res*., **2**, No. 1, 433–435 (2011), www.biomedscidirect.com.
12. Int. Conf. Harmonization Guidelines, Validation of Analytical Procedures: Text and Methodology Q2(R1).
13. B. Babu, S. N. Meyyanathan, B. Gowramma, S. T. Narenderan, *Int. J. Chem. Tech. Res*., **10**, No. 15, 183–188 (2017), www.sphixsai.com.