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Analytical Method Development and Validation for the Estimation of Erlotinib Hydrochloride by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Erlotinib hydrochloride by using the HPLC method for Erlotinib hydrochloride as per ICH Guide lines. Shimadzu LC-2010 series with UV Detector and ACE 3 C18 (150x4.6mm, 3µ) column, injection of 10 µl is injected and eluted with the Preparation of mobile phase A: Mixed well about 930 mL of purified water, 70 mL of tetrahydrofuran and 1.5 mL of Trifluroacetic acid. And Preparation of mobile phase-B: Mixed well about 450 mL of purified water, 480 mL of acetonitrile, 70 mL of tetrahydrofuran and 1.5 mL of Trifluroacetic acid in the ratio 65:35, which was pumped at a flow rate of 1.5 ml at 245 nm. The peak of Erlotinib Hydrochloride was found well separated at 6.1 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, linearity, rang, accuracy, precision, specificity, ruggedness, and robustness. UV-spectroscopy (made- Shimadzu) was used with mobilphse-A and mobile phase-B in the ratio 65:35, which was detected at single point 245 nm. The absorbance of Erlotinib Hydrochloride was found. The analytical method validation of Erlotinib Hydrochloride by RP-HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Erlotinib Hydrochloride.

Keywords: Erlotinib hydrochloride, HPLC, Rang, Accuracy, Precision, Specificity, Ruggedness

A R T I C L E I N F O

CONTENTS

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1. Introduction

Analytical methods: Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1,2]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [5, 6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

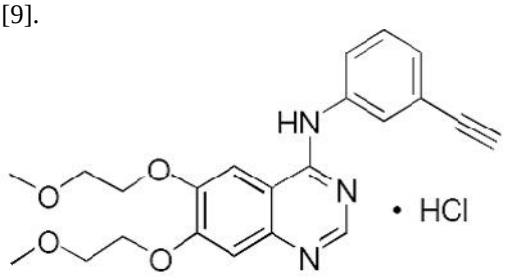

Figure 1: Erlotinib hydrochloride

2. Materials and Methods

Apparatus: The instrument used was HPLC-auto sampler –UV detector, Separation module 2695, photo diode array detector 996, Empower-software version-2.

Reagents and Materials

The solvents used were Acetonitril, Tetrahydrofuran, Trifluroacetic acid, Hydrochloric acid, Hydrogen peroxide and Water [10].

Selection of detection wavelength:

The Appropriate dilutions of the standard drug solutions were prepared for 10 ppm of Erlotinib in Water + ACN (50: 50) as a diluent. Erlotinib Solution was scanned using double beam UV- VIS spectrophotometer between the ranges of 200 to 400 nm. After scanning the drug, λ_{max} for Erlotinib was found to be 245 nm. Hence this wavelength has been selected for the complete experiment [11].

Optimization Chromatographic trials for Estimation of Erlotinib hydrochloride by RP- HPLC.

Optimization Chromatographic conditions

Column : ACE 3 C_{18}, 150 X 4.6 mm, 3 µm
Mobile phase ratio: Mobile phase A: Mobile phase B (65:35)
Detection wavelength: 245 nm
Flow rate : 1.5 ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10mins

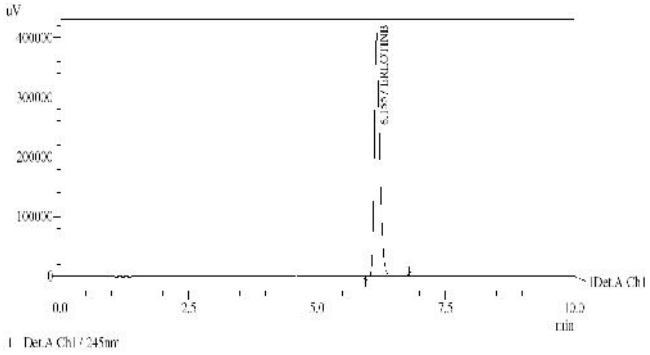

Figure 2: Optimization Chromatogram

Observation: The retention of peak was good, resolution was good, tailing factor was less than 2, theoretical plates were more than 2000, and this trial was taken as optimized method.

Procedure

Preparation of standard solution (100 µg/ml):

Accurately weigh and transfer about 27.30 mg of Erlotinib HCl standard into 50 ml volumetric flask to this added about 15 ml of diluent and sonicated for about 2 minutes with occasional shaking. Make up to the volume with diluent. Pipette out 5.0 ml of the above stock solution into 25 ml volumetric flask and made up to the volume with diluent, mix well. Filter a portion of solution through 0.45µm GHP membrane filter and inject into HPLC [12].

Preparation of sample solution (100 µg/ml):

Average weight: 467.42 mg
Sample weight: 632.13 mg

Grind 10 tablets by using mortar and pestle and accurately weighed and transferred about 632.13 mg of powdered sample into 200 ml volumetric flask to this added about 150 ml of diluent and sonicated for about 15 minutes with occasional shaking. Make up to the volume with diluent mixed well. Filter a portion of solution

through 0.45µm GHP membrane filter and pipette out 5.0 ml of the above filtered solution into 50 ml volumetric flask and made up to the volume with diluent, and inject into HPLC[13].

3. Results and Discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank [14].

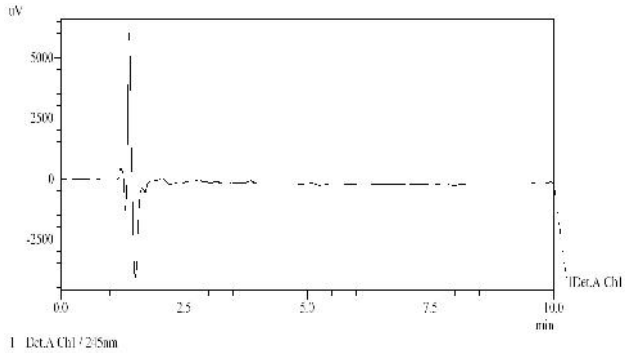

Figure 3: Chromatogram of Blank

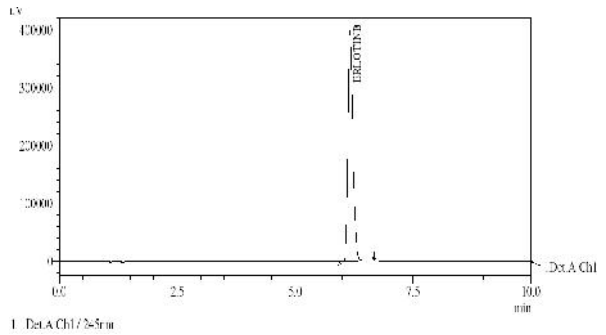

Figure 4: Chromatogram of Sample

2. Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Linearity shall be established by demonstrating that the area response obtained is directly proportional to the concentration of standard solution. The standard solutions are to be prepared at six different concentration levels ranging from 10 % to 200 % of working concentration and finding the response at each concentration level for assay and the results are observed. The linearity study was performed for the concentration of 10 ppm to 200 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [15].

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

The linearity study was performed for the concentration of 10ppm to 200ppm. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [16].

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

Percentage method:

For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively [17].

Acceptance criteria: The mean % recovery of the Erlotinib hydrochloride 100.8 should be not less than 95.0% and not more than 105.0%.

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported [18].

Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [19].

Intermediate Precision:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [20].

Validation of the Method

Linearity: The linearity study was performed for the concentration of 10 ppm to 200 for Pantoprazole and chromatograms are shown below [21].

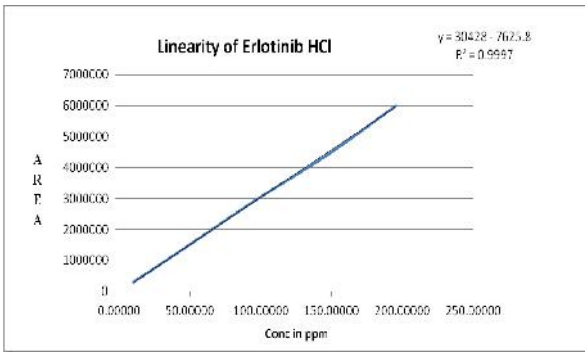

Figure 5: Calibration graph of Erlotinib hydrochloride

Table 1: Calibration data of Erlotinib hydrochloride

Levels	Conc. (µg/ml)	Response (mean area)
Level 1 (10%)	9.8	314792
Level 2 (25%)	24.45	750190
Level 3 (50%)	48.98	1500376
Level 4	97.80	3000502
Level 5	146.66	4400702
Level 6	195.55	6000703

Y – intercept	7625.9
Slope	30428
Correlation Coefficient	0.9999

Recovery Studies: Sample solutions at different conc. (50%, 100%, and 150%) were prepared and the % recovery was calculated [22].

Table 2: Accuracy results for Erlotinib hydrochloride

Recovery Level	**% Mean Recovery**
Recovery level 50%	99.80
Recovery level 100%	100.2
Recovery level 200%	101.7
Mean Recovery	100.6
SD	1.00
% RSD	1.00

Table 3: System suitability parameters for Erlotinib hydrochloride

System suitability parameters	**Acceptance criteria**
Tailing factor	NMT 2.0
Theoretical plate	More than 6000
% RSD	NMT 2.0

Table 4: Repeatability results for Erlotinib hydrochloridee

Sample no	**Sample area**	**% Assay**
Test-1	3081709	101.4
Test-2	3008337	99.4
Test-3	3005237	100
Test-4	3011709	99.6
Test-5	3005009	99.8
Test-6	3015503	100.4
	Average	100.1
	SD	0.69
	%RSD	0.689

Table 5: Intermediate precision for Erlotinib hydrochloride

Sample No	**Mean area**	**% Assay**
Test-1	3054075	101.1
Test-2	3012937	100.1
Test-3	3015385	100.6
Test-4	3111163	100
Test-5	3081709	101.9
Test-6	3100229	100.7
	Average	100.7
	SD	0.7
	%RSD	0.7

4. Conclusion

A HPLC method for Erlotinib hydrochloride was developed and validated in tablet dosage form as per ICH Guide lines. Shimadzu LC-2010 series with UV Detector and ACE 3 C18 (150x4.6mm, 3µ) column, injection of 10 µl is injected and eluted with the Preparation of mobile phase A: Mixed well about 930 mL of purified water, 70 mL of tetrahydrofuran and 1.5 mL of Trifluroacetic acid. And Preparation of mobile phase-B: Mixed well about 450 mL of purified water, 480 mL of acetonitrile, 70 mL of tetrahydrofuran and 1.5 mL of Trifluroacetic acid in the ratio 65:35, which was pumped at a flow rate of 1.5 ml at 245 nm. The peak of Erlotinib Hydrochloride was found well separated at 6.1 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, linearity, rang, accuracy, precision, specificity, ruggedness, and robustness. UV- spectroscopy (made- Shimadzu) was used with mobil phse-A and mobile phase-B in the ratio 65:35, which was detected at single point 245 nm. The absorbance of Erlotinib Hydrochloride was found. The analytical method validation of Erlotinib Hydrochloride by RP-HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Erlotinib Hydrochloride.

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