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Original Research Article

Development and validation of RP-HPLC and UV method for erlotinib hydrochloride tablets

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ABSTRACT

A simple, sensitive, precise, selective reverse phase high performance liquid chromatographic method was developed and validated for erlotinib hydrochloride in tablet dosage form.(0.02M)The separation was achieved on C18 column (150mm×4.6mm.i.d.,5.0μm) using potassium dihydrogen phosphate: acetonitrile in the ratio 50:50v/v as mobile phase having pH 4.5 was adjusted with methanol and flow rate 1ml/min. Detection was carried out using a UV detector at 248nm. The column temperature was adjusted at 30^{o}C. The method was validated for precision, linearity and range, stability and robustness. The developed and validated method was successfully applied for the quantitative analysis of ERLONAT tablets. The total chromatographic analysis time per sample was about 7min with Erlotinib eluting at 6.547min.Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curves were linear over the concentration ranges, 88.32- 132.48μg/ml for erlotinib. The high recovery confirms the suitability of the proposed method for the determination of Erlotinib in ERLONAT tablets. The results of analysis have been validated according to ICH guideline requirements. The method can be applied for Erlotinib hydrochloride tablets.

1. Introduction

Erlotinib, N-(3-ethynylphenyl)-6,7-bis (2-methoxyethoxy)-4-quinazolinamine (Figure 1), is a new drug for the treatment of lung cancer.[1] Simultaneous quantification of Erlotinib, Gefitinib and Imatinib in human plasma by liquid chromatography tandem mass spectrometry, therapeutic drug monitoring. The mechanism of action involved is an epidermal growth factor receptor inhibitor. It specifically targets the epidermal growth factor receptor (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. It binds in a reversible fashion 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. These then use the molecule of ATP to autophosphorylate each other, which causes a conformational change in their intracellular structure, exposing a further binding site for binding proteins that cause a signal cascade to the nucleus. By inhibiting the ATP, autophosphorylation is not possible and the signal is stopped.[2]

Simultaneous quantification of erlotinib, gefitinib and imatinib in human plasma by liquid chromatography, tandem mass spectrometry.[3] High performance thin layer chromatographic method for estimation of erlotinib hydrochloride as bulk drug.[4] A Simple HPLC –UV method for the simultaneousquantification of gefitinib and erlotinib in human plasma.[5] Separation and determination of process- related impurities of Erlotinib using reverse phase

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HPLC with a photodiode array detector.[6] New derivative spectrophotometric methods for the determination of erlotinib hydrochloride (A tyrosine kinase inhibitor).[7] Development and validation of a stability indicating HPLC method for the quantification of impurities in erlotinib hydrochloride dosage forms.[8] Validation of HPLC method for determination of erlotinib related substance in pharmaceutical dosage form.[9] Analytical method development and validation of erlotinib hydrochloride in bulk and pharmaceutical dosage form by RP-HPLC.[10] So we planned to develop UV and RP- HPLC method and to validate developed method according to ICH norms.[11–13]

2. Materials and Methods

2.1. Equipment and Apparatus used in HPLC

Analytical balance (Metller Toledo AG-245), Waters LC system equipped with 2695 pump and UV visible detector, Chromatographic data software : EMPOWER, ColumnC18 , 150mm×4.6mm. 5.0μ column, Vacuum filter pump, Ultrasonicator (sonarex), Membrane filter (0.45 and 0.2microns), pH-Meter (Lab India), Model: LC 2010 (Shimadzu).

2.2. Chemicals and Reagents used for HPLC

Acetonitrile (HPLC grade), Orthophosphoric acid (HPLC grade), Potassium dihydrogen phosphate.-buffer. (HPLC grade), Water (HPLC grade), Methanol (HPLC grade), Hydrochloric acid (HPLC grade),Sodium hydroxide (HPLC grade), Hydrogen peroxide (HPLC grade). Purchased from Sd fine-chem Ltd., Mumbai, India. High purity deionized water was obtained by double distillation and purification through milli-Q water purification system. The 0.45 μm nylon filter was purchased from advanced micro device Pvt Ltd., Chandigarh.

2.3. Equipment and Apparatus used in UV

Analytical balance (metller toledo AG-245), Shimadzu UV visible detector, Model: UV 1700, Ultrasonicator (sonarex), pH meter (lab India).

2.4. Chemicals and reagents used for UV

Methanol, Water, Hydrochloric acid.

2.4.1. Buffer preparation

Potassium dihydrogen orthophosphate (2.72 g) was dissolved in 100 mL of HPLC grade water and pH 5.0 was adjusted with orthophosphoric acid. It was filtered through 0.45 μm nylon membrane filter and degassed. It was used as a diluent for the preparation of sample and standard solution.

2.5. Method

2.5.1. Selection of wavelength

Accurately weighed quantity of the sample equivalent to 50 mg of Erlotinib was taken in a 50 mL volumetric flask. 25 mL of methanol was added, sonicated for 10 min and filtered through 0.45 μm nylon filter. The above solution was scanned between 200 and 400 nm by UV spectroscopy (Figure 2).

2.6. Method development by UV-spectroscopy

The identification method for erlotinib hydrochloride in erlotinib hydrochloride tablets by UV-VISIBLE spectrophotometer test has been checked for specificity with respect to placebo interference and also to confirm the identification of sample by comparison with maxima obtained from reference standard.

2.7. Methology

2.7.1. Preparation of Diluent (0.1M Hydrochloric acid)

Dissolve 8.5ml of hydrochloride acid in 1000ml of water and mix.

2.7.2. Preparation of standard solution

Weigh and transfer accurately about 54.8mg of erlotinib hydrochloride into 100ml clean, dry volumetric flask, add 10 ml of methanol and add diluent 0.1M HCL to makeup the volume upto 100ml.

2.7.3. Preparation of Sample solution

Weigh and transfer accurately about 170.9mg of erlotinib hydrochloride tablet powder into 100ml clean, dry volumetric flask, add diluent 0.1M HCL makeup to 100ml . Then take 2ml of that solution to dilute 0.1M HCL make up to 100ml.

2.7.4. Procedure

Record the UV absorbance spectrum of the standard and sample solution between 200 to 400nm using diluent in 1cm cell. Erlotinib hydrochloride shows maximumabsorbance at 248nm by using 0.1M HCL.

2.8. Method validation by UV-spectroscopy

The analytical procedure are validated for the following parameters as per ICH Guidelines and reported.

2.9. Validation parameters

2.9.1. System Suitability

To verify the analytical system is working properly and can give accurate precise results, the system suitability parameters are to be set. Calculation shown in (Table 3).

The percentage relative standard deviation should not more than 2.

2.9.2. Linearity

Linearity shall be established by demonstrating that the absorbance obtained is directly proportional to the concentration of the standard solution. The standard solutions are to be prepared at 5 different concentration levels ranging of working concentration and finding the response at each concentration level for assay.

2.9.3. Preparation of standard solution

54.8mg of erlotinib is dissolved in 10ml of methanol. Then add 0.1M HCL upto 100ml in the volumetric flask.

Corelation coefficient (r) of Erlotinib was found to be 0.9987.

2.10. System precision

54.8 mg of erlotinib powder is dissolved in 10ml of methanol. Then makeup the volume up to 100ml. Calculation shown in (Table 4). The percentage relative standard deviation of Erlotinib was found to be less than2.0

2.11. Recovery studies

The stock solution was diluted with mobile phase. Further to obtain a concentration ranging from 80%, 100%, 120%. Calculation shown in(Table 5) The percentage recovery of Erlotinib was found to be 78.39 to 117.83.

2.11.1. Chromatographic conditions

The mobile phase containing Acetonitrile: Potassium dihydrogen phosphate (buffer 0.02M) PH 4.5 with methanol in the ratio of 50:50v/v were filtered through 0.45μm nylon filter ,degassed and were pumped from the solvent reservoir in the column. The flow rate of mobile phase was maintained at 1ml/min and detection wavelength was set at 248nm with a run time of 7min. The column and the HPLC system were kept in 30^{o}C temperature.

2.11.2. Preparation of diluents

The mixture of acetonitrile and potassium dihydrogen phosphate (0.02M) in the ratio of 50:50(v/v) used as diluents for the study. The chromatogram of the blank is presented.

2.11.3. Preparation of standard stock solution

About 55 mg of working standard of Erlotinib Hydrochloride was weighed and transferred into a clean and dry 50 ml standard flask, the sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase filtered through 0.45μ filter.then.5 ml of the stock solution was pipetted into a 50 ml standard flask and diluted to mark with mobile phase. The retention time of Erlotinib Hydrochloride was found to be 6.545 min when injected and chromatograms are shown.

2.11.4. Preparation of sample solution

Average weight of the tablet was computed from the weight of 10 tablets. The tablets were powdered. The tablet powder equivalent to 170.9mg of Erlotinib was accurately weighed and transferred into a clean and dry 100 ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase. The solution was filtered by using Whatmann filter paper. (Concentration 1000μg/ml).5 ml of the stock solution was pipetted into a 50 ml standard flask and diluted to mark with mobile phase. It was filtered through 0.45μ filter (Concentration-50 mcg/ml). The sample was injected and chromatograms were recorded and shown. The amount of Erlotinib present in each tablet formulation was calculated by comparing the peak area of the test with that of the standard.

2.12. Method validation by HPLC

Validation of analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application.

2.13. Validation parameters

2.13.1. System suitability

System suitability of the method was performed by calculating the parameters namely, tailing factor and number of theoretical plates on replicate injection of standard solution.

Tailing factor = 1.00

No. Of. Theoretical plate = 11269.63

2.13.2. Acceptance criteria

Tailing factor should be not more than 2.

No of theoretical plate should be not less than 2000.

2.13.3. Linearity report for erlotinib

Linearity was assessed by performing measurement at several analyte concentrations. A minimum five concentrations were recommended for linearity studies. The linearity of an analytical method is its ability to show test results that is directly proportional to the concentration of analyte in sample with in a given range. The linearity of an analytical method was determined by mathematical treatment of test result obtained by analysis of samples with analyte concentration across claimed range of peak area Vs concentration is plotted and percentage curve fitting is calculated.Calculation shown in (Table 5)

2.13.4. System precision for Erlotinib

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. Calculation shown in (Table 4)

2.13.5. Method precision of Erlotinib

The sample 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. Calculation shown in (Table 7)

2.14. Recovery studies

The stock solution was diluted with mobile phase. Further to obtain a concentration ranging from 50%, 100%, 150%.Calculation shown in (Table 8).

2.14.1. Robustness

1. **Effect of variation of** $_{pH:}$A study was conducted to determine the effect of variation in +0.2 pH and -0.2 $_{pH}$. Sample solution was prepared and injected into the HPLC system. The values were measured and shown in(Table 9)
2. **Effect of variation of temperature:** A study was conducted to determine the effect of variation in temperature Standard solution was prepared and injected into the HPLC system. The effects of variation in temperature were measured and shown in (Table 10).

The percentage relative standard deviation was found to be not more than 2.

*2.14.2. **Degradation Studies***

5ml of standard stock solution was mixed with 5ml of degradation agents then make up the volume with 50ml of the mobile phase. Actual amount of erlotinib was found to be 98.3. Calculation shown in (Table 11)

Addtion of 3% hydrogen peroxide.

Addition of 0.1M HCL.

Addition of 0.1M NaoH.

Degradation limit 5 to 10%.

2.14.3. Limit of Detection (LOD)

The LOD was determined by the formula: **LOD = 3.3** σ**/ S**

Where

σ = Standard deviation of the response

S = Slope of calibration curve

LOD = 3.3 (0.00896/38)

LOD = 0.000766 μg/ml

From the formula limit of detection was found to be = 0.000766 μg/ml.

2.14.4. Limit of Quantification (LOQ)

Quantitation limit (QL) may be expressed as: **LOQ=10**σ**/S**

Where

σ = Standard deviation of the response

S =S of calibration curve

LOQ = 10 (0.00896/38.56)

LOQ = 0.002323 μg / ml.

From the formula limit of quantitation was found to be = 0.002323 μg / ml.

3. Results and Discussion

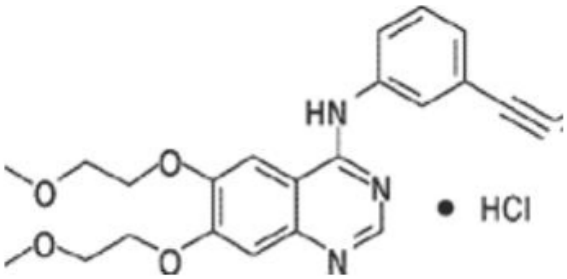

Fig. 1: Chemical structure of Erlotinib hydrochloride.

3.1. RP-HPLC method. (Tables 5, 6 and 7)

3.1.1. Recovery studies.(Tables 8, 9, 10 and 11)

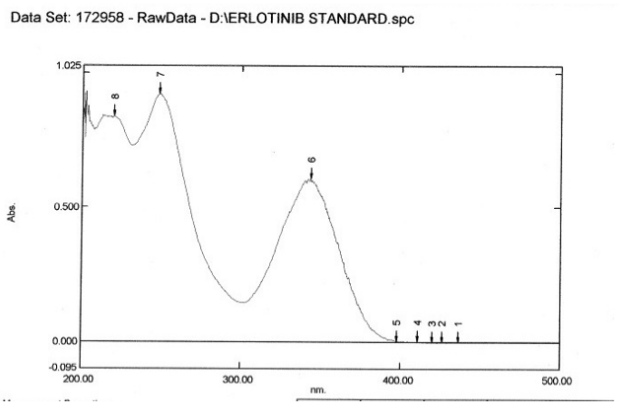

Fig. 2: Chromatogram of erlotinib for standard solution in UV method**.**

4. Discussion

The observations and results obtained for HPLC validation parameter including Linearity and Range, Precision (System precision, Method precision) and Robustness

Parameters	**Limit**	**Observations**	**Result**
System suitability	%RSD=not more than 2.0	%RSD=1.23	Passes.
Linearity	Correlation of coefficient (R) = 0.999	Correlation of coefficient (R) = 0.998.	Passes
Precision	%RSD = not more than 2.0	Within the limits	Passes
Recovery studies	80% to 120%	78.39 to 117.83%	Passes

Table 1: Method validation report by RP-HPL

Parameters	**Limit**	**Observations**	**Result**
Linearity	Correlation coefficient = Not more than 0.999	Erlotinib hydrochloride (r) = 0.998	Passes
System precision	%RSD= Not more than 2.0	% RSD = 0.0913	Passes
Method precision	%RSD = Not more than 2.0	%RSD = 0.2320	Passes
Robustness	%RSD= Not more than 2.0.	Within the limits.	passes
	50%	50% = 49.49%	
Recovery studies	100%	100%= 99.18%	passes
	150%	150%= 148.56%	
Degradation studies	Limit 5 to 10%	Within the limits.	Passes.
LOD	Not more than two	0.00766μg/ml	Passes
LOQ	Not more than ten	0.002323 μg/ml	Passes

Table 2: System suitability in UV method

Sample name	%RSD
Blank(diluent)	——
Standard solution	1.23

Table 3: Linearity in UV method

Concentration	**Absorbance**	**Mean**	**SD**	**%RSD**
5.48	0.475	0.4868	0.0012	0.2465
8.22	0.689	0.6401	0.0041	0.6405
10.96	0.918	0.9063	0.0102	1.1254
13.7	1.142	1.1293	0.01616	1.4309
16.44	1.331	1.297	0.0231	1.7810

Table 4: Percentage Recovery data forErlotinib in UV method.

Concentration in %	**Absorbance**	**%Recovery**
80%	0.899	78.39
100%	0.840	99.18
120%	0.998	117.83

Table 5: Linearity Report forErlotinib

Parameters	**Results**
Slope	38.56
Intercept	78.40
Correlation co-efficient	0.999
Percentage curve fitting	99.9%

Table 6: Precision data of the system

Injection no	**Peak area**	**%Recovery**
1	5490217	98.52
2	5485395	98.02
3	5497965	98.04
4	5496878	98.22
5	5496512	98.34
6	5490079	98.20

Mean = 5492841. SD = 5019.67. The percentage related standard deviation of Erlotinib was found to be 0.0913%.

Table 7: Method precision ofErlotinib

Injection no	Peak Area	% Recovery
1	5022126	98.28
2	5015948	98.16
3	5046105	98.75
4	5018685	98.22
5	5017221	98.19
6	5039165	98.62

Mean = 98.37. SD = 0.2505. The percentage related standard deviation was found to be 0.2546.

Table 8: Percentage Recovery data forErlotinib

S.No	Spike level	Peak area	%Recovery	SD	Mean	%RSD
1	50%	5061114	49.52			
	50%	5056333	49.47	0.0251	49.49	0.0507
	50%	5058642	49.50			
2	100%	5068235	99.19			
	100%	5068093	99.18	0.0057	99.18	0.0057
	100%	5067989	99.18			
3	150%	50031421	148.74			
	150%	51020120	149.77	1.3093	148.56	0.8813
	150%	50132100	147.17			

Table 9: Robustness (change in $_{p}$ **H)**

Changes	Peak area	% Recovery	SD	Mean	%RSD
+0.2$_P$H	5091247	99.64			
+0.2$_P$H	5076577	99.35	0.03535	99.49	0.035
-0.2$_P$H	5018994	98.22			
-0.2$_P$H	5028789	98.41			
-0.2$_P$H	5053805	98.90	0.5419	98.74	0.5488

Table 10: Robustness (change in temperature)

Changes	Peak area	% Recovery	SD	Mean	%RSD
+2 temp	5050790	98.84			
+2 temp	5055938	98.94			
+2 temp	5461690	99.06	0.1101	98.94	0.112
-2 temp	5089248	99.43			
-2 temp	5020402	98.25			
-2 temp	5065119	99.12	0.6117	98.93	0.6183

Table 11: Degradation studiesAddtion of 3% hydrogen peroxide.

Area	**%Recovery**	**%degradation**
5023521	98.31	0.06
5012520	98.09	0.28
5021432	98.27	0.10
Addition of 0.1M HCL.		
Area	**%Recovery**	**%degradation**
5011220	**98.07**	**0.30**
5021340	**98.07**	**0.30**
5095026	**98.05**	**0.32**
Addition of 0.1M NaoH.		
Area	**%Recovery**	**%degradation**
5013127	98.11	0.26
5098410	98.18	0.19
5093982	98.20	0.17

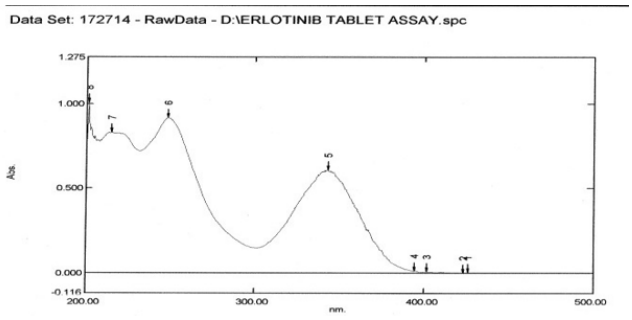

Fig. 3: Chromatogram of erlotinib for sample solution in UV method.

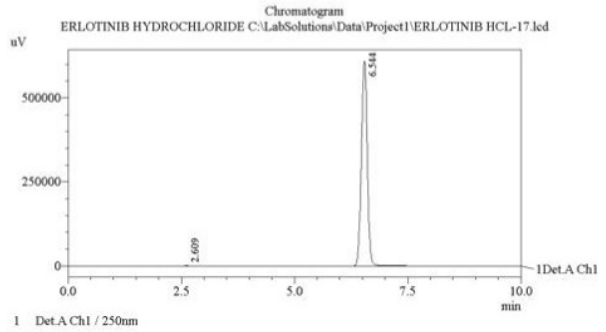

Fig. 4: Chromatogram of erlotinib for standard solution in RP-HPLC method.

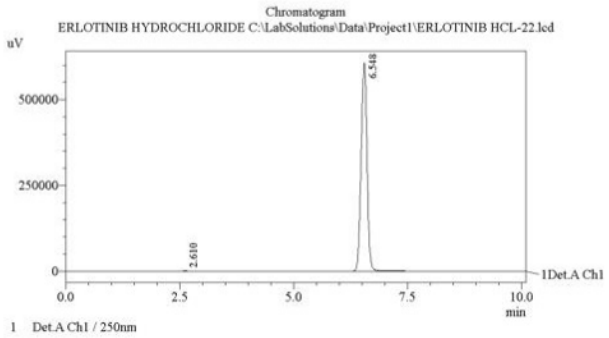

Fig. 5: Chromatogram of erlotinib for sample solution in RP-HPLC method.

within the acceptance criteria. Since the results are within acceptance criteria for all validation parameters, therefore, the method is considered as validated and suitable for intended use.

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

Erlotinib was slightly soluble in methanol and very slightly soluble in water. Methanol and Mixture of Buffer was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 88.32-132.48 μg /ml.

The correlation co-efficient of the Erlotinib was found to be 0.999. The system precision of Erlotinib was found within the limit. The percentage relative standard deviation was found to be 0.0913. The method precision of Erlotinib was found to be within the limit. The relative standard deviation was found to be 0.2546.

The % recovery of Erlotinib was found to be in the range of 50%, 100%, 150%. As there was no interference due to excipients and mobile phase, the method was found to be specific.50% recovery of Erlotinib- percentage relative standard was found to be 0.0507.100% recovery of Erlotinib -percentage relative standard was found to be 0.0057.150% recovery of Erlotinib – percentage relative standard was found to be 0.8813.

The percentage degradation should be in 5 to 10%. The degradation studies of Erlotinib was found to be within the limits. The method was robust as observed from insignificant variation in the results of analysis by changes in pH and temperature separately and analysis being performed .The observation and results obtained for UV validation parameters including system suitability, linearity, precision, recovery within the acceptance criteria.

In system suitability, the percentage relative standard deviation was found to be 1.23.The correlation of coefficient of Erlotinib was found to be 0.998. In precision, percentage relative standard deviation was found to be less than 2. (0.2465 to 1.7810). The acceptance criteria of percentage recovery was 80% to 120%. The percentage recovery of Erlotinib was found to be the range of 78% to 117%.

5. Summary and Conclusion

A HPLC and UV method for Erlotinib hydrochloride was developed and Validated in tablet dosage form as per ICH guidelines. This condition is applied only for tablet dosage form. Good agreement was seen in the assay results of pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Erlotinib in the pharmaceutical formulation.

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hydrochloride drug and providing the facilities necessary to carry out research work.

7. Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

8. Source of Funding

None.

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