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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF ERLOTINIB HYDROCHLORIDE

U. Mohan Kumar[1], P. Sravanthi[2], S. Lakshmi Naga Aparna[3], G. Tejaswi[4] and I. Theja[5*]

[1]Professor, Nirmala College of Pharmacy, Kadapa, Andhra Pradesh, India.

[2,3]Assistant Professor, Nirmala College of Pharmacy, Kadapa, Andhra Pradesh, India.

[4]Assistant Professor, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak District, Telangana, India.

[5*]Associate Professor, Nirmala College of Pharmacy, Kadapa, Andhra Pradesh, India.

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***Corresponding Author**
I. Theja
Associate Professor, Nirmala College of Pharmacy, Kadapa, Andhra Pradesh, India.

ABSTRACT

In the present study, a reliable and efficient reverse-phase high-performance liquid chromatography (RP-HPLC) method was established for the quantitative estimation of Erlotinib Hydrochloride. Chromatographic separation was achieved using a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm), with a mobile phase composed of acetonitrile and phosphate buffer in an 80:20 (v/v) ratio. The pH of the buffer was adjusted to 5.0 using orthophosphoric acid. The analysis was carried out at a flow rate of 1.0 mL/min, and detection was performed at 245 nm. Erlotinib exhibited a retention time of 3.7 minutes, confirming the rapidity of the method. The calibration curve showed good linearity within the concentration range of 1–6 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.01 ng/mL and 1 ng/mL, respectively. Method validation was conducted following ICH Q2(R1) guidelines, demonstrating its accuracy, precision, and suitability for the intended purpose. This validated method was effectively applied for the routine estimation of Erlotinib HCl in both bulk drug and pharmaceutical formulations.

KEYWORDS: Erlotinib HCl, HPLC, Validation, ICH Guidelines, Method development.

INTRODUCTION

Erlotinib, N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy) quinazolin-4-amine (Fig.1), is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor used in the treatment of non-small cell lung cancer, pancreatic cancer, and several other cancers.[1] It is usually marketed under the trade name Tarceva. Erlotinib reversibly binds to the epidermal growth factor receptor (EGFR) tyrosine kinase at the adenosine triphosphate (ATP) binding site of the receptor.[2,3] Recent studies show that Erlotinib is also a strong inhibitor of JAK2V617F, a mutated tyrosine kinase JAK2 present in the majority of patients with polycythemia vera (PV) and a large percentage of patients with idiopathic myelofibrosis or essential thrombocythemia. This discovery brings to light the possible application of Erlotinib in the treatment of JAK2V617F-positive PV and other myeloproliferative disorders.[4] Numerous analytical techniques have been documented for the quantitative determination of Erlotinib in pharmaceutical formulations, with the majority of reported HPLC methods demonstrating retention times exceeding 4 minutes.[5] Despite the availability of these methods, no reports to date have documented an approach achieving a retention time below 3 minutes. This highlights the need for a more time-efficient method without compromising accuracy or reliability. In response to this gap, the present study focuses on the development of a simple, precise, and rapid RP-HPLC method for the estimation of Erlotinib, achieving a retention time of less than 4 minutes. This optimization not only reduces analysis time but also enhances the suitability of the method for routine quality control applications.

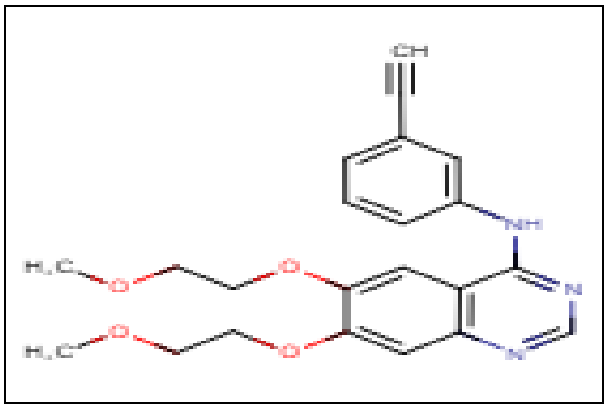

Fig.1: Chemical Structure of Erlotinib.

MATERIALS AND METHODS

Instruments

HPLC system (Shimadzu, SPD20A LC20AT, Japan) equipped with a manual sampler. Detection of analytes was done by using a UV/Vis detector. Chromatographic data were

acquired by using Spinchrom software. An analytical balance (Shimadzu, BL-220H, Japan), a Phenomenex C_{18} column (250mm×4.60mm×5µm), and a Hamilton sample injector were used for the study.

Reagents and chemicals

Erlotinib hydrochloride was procured from Yarrowchem, Mumbai. HPLC grade water and acetonitrile, and methanol were procured from Hiedia, Mumbai. Sodium dihydrogenphosphate, orthophosphoric acid, and triethanolamine were purchased from Himedia Laboratories Pvt. Ltd. (Mumbai)

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, filtered through a 0.45 µm nylon membrane filter, and degassed by ultrafiltration. It was used as a solvent for the preparation of the sample and standard solution.

Preparation of standard solution

A standard stock solution of ERL was prepared by weighing and transferring 10mg of the drug into 10ml volumetric flask. The substance was slightly dissolved with 5ml DMSO, and the volume was made up with the mobile phase to attain a concentration of 1000µg/mL.

Selection of wavelength

Standard solution of Erlotinib was scanned in the UV spectral range of 200-400nm. The spectrum shows a maximum wavelength at 245 nm, as shown in Fig.2.

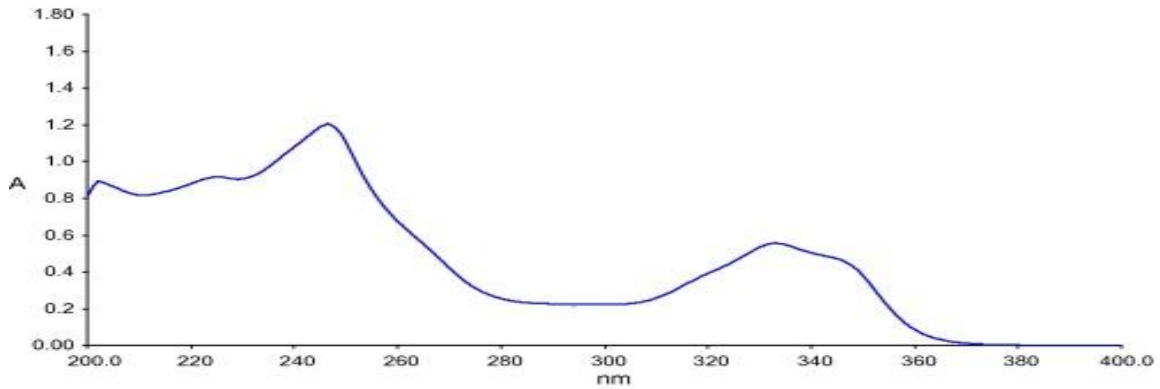

Fig. 2: UV Spectra of Erlotinib HCl.

Method development trials

Trail 1

Table 1: Method development trial 1.

S. No	Parameter	Condition
1	Mobile Phase	45:55 v/v (Phosphate buffer: Acetonitrile)
2	Stationary phase	phenomenex - C18 column
3	Flow rate	1.0 ml/min
4	pH	7.4
4	Run time	6 mins

Trail 2

Table 2: Method development trial 2

S. No	Parameter	Condition
1	Mobile Phase	80:20 v/v (Phosphate buffer: Acetonitrile)
2	Stationary phase	phenomenex - C18 column
3	Flow rate	1.0 ml/min
4	pH	5.0
4	Run time	5 mins

Method validation

The method was validated following ICH recommendations Q2R1. System suitability, linearity, accuracy, precision, LOD & LOQ, and robustness are among the validation parameters.[6,7,8]

RESULTS AND DISCUSSION

Trail 1

Under these conditions, multiple broad peaks were observed. The chromatogram shows peaks corresponding to Erlotinib, but the peak is not symmetrical. Therefore, the conditions were not suitable for the separation and analysis of Erlotinib. The chromatographic results indicate that the mobile phase used in this trial was not suitable for resolving the analytes. Consequently, further study was conducted with a change in the mobile phase while keeping the same column.

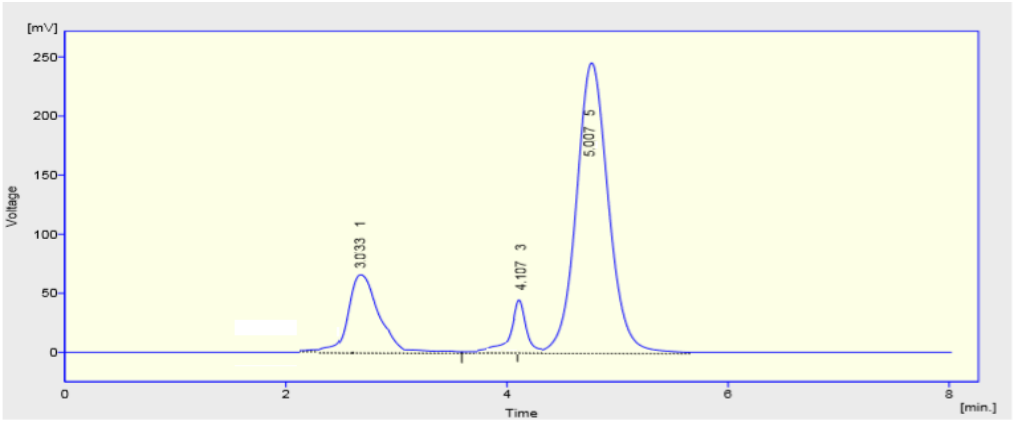

Fig. 3: Representative chromatogram of Erlotinib HCl.

Trail 2

Under the specified chromatographic conditions, a single sharp and symmetrical peak for Erlotinib was observed at a retention time of 3.717 minutes. The peak exhibited minimal tailing and a strong response, indicating improved resolution and suitability of the selected mobile phase for Erlotinib analysis.

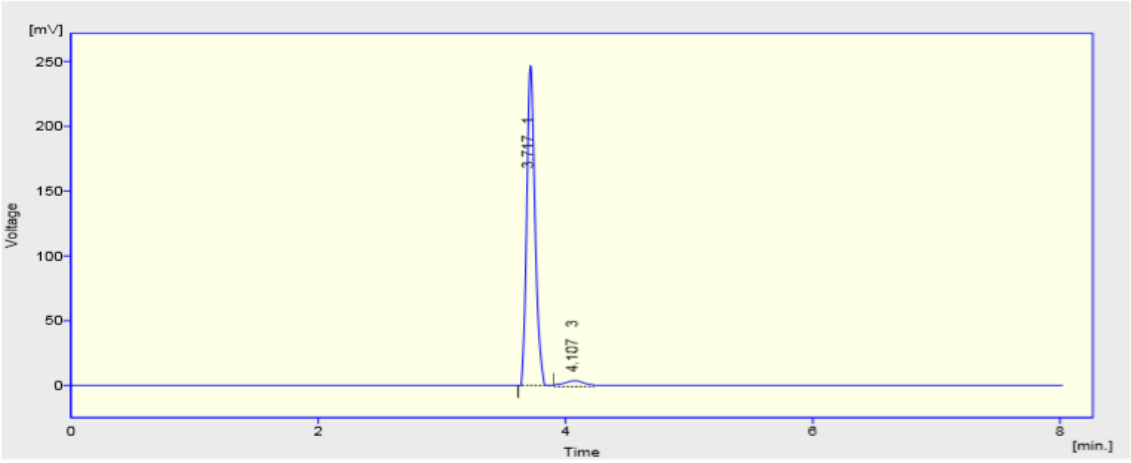

Fig 4: Representative Chromatogram for Erlotinib HCl

In the optimized conditions, well-resolved, retained, and accepted system suitability was observed. The optimized conditions are given in Table 3.

Table 3: Optimized Chromatographic conditions.

S. No	Chromatographic Parameters	Results
1	Mobile Phase (v/v)	80:20 v/v (Phosphate buffer: Acetonitrile)
2	Stationary Phase	Inertsil Octa Decyl Silane (ODS)- Phenomenex C_{18} column (250cm×4.60mm×5µm)
3	pH	5.0
4	Flow Rate	1 mL/min

5	Wavelength	245 nm
6	Pump mode	Isocratic
7	Runtime	5 mins
8	Retention time	3.7 min

Method validation [9, 10, 11]

System suitability parameters:

The system suitability parameters were assessed by making standard solutions of Erlotinib (3µg/ml) and injecting them six times. All the system's appropriate parameters were passed and remained within the limitations.

Table 4: System Suitability.

S.NO	Concentration (µg/ml)	Retention time	Peak area	Mean	SD	%RSD
1	3 µg/ml	3.34	1073.005	1069.391	15.5624	1.455
2		3.34	1057.775			
3		3.34	1050.71			
4		3.33	1064.68			
5		3.33	1075.116			
6		3.35	1095.062			

Linearity

The linearity of the calibration curve was determined over the concentration range of 1-6 µg/ml. The correlation coefficient obtained was 0.995. The results were shown in Table 5 and Figure 5.

Table 5: Linearity studies for Erlotinib HCl.

Concentration (µg/ml)	Peak area (n=3)
1	363.364 ± 37.156
2	657.119 ± 47.184
3	1074.003 ± 59.451
4	1330.234 ± 62.543
5	1636.569 ± 53.267
6	2127.432 ± 71.223

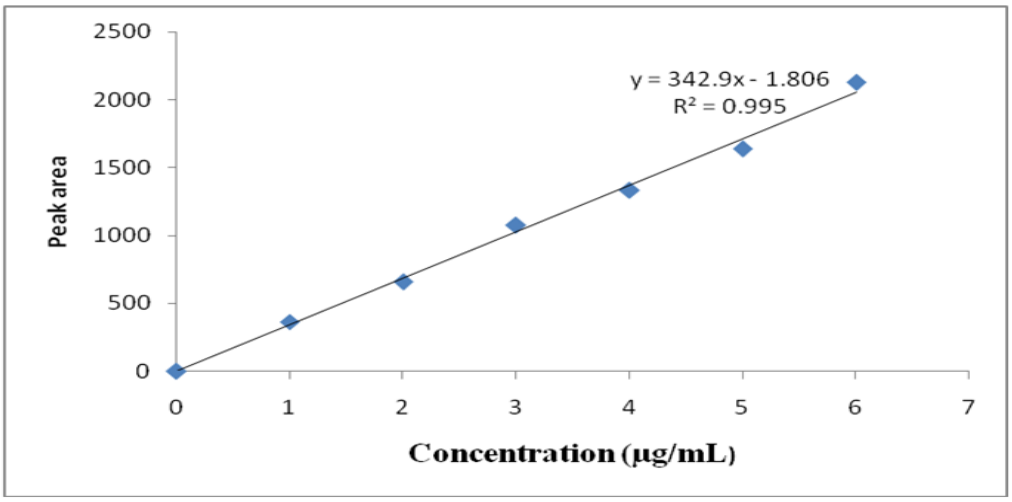

Fig. 5: Calibration curve of Erlotinib HCl.

Precision

Precision was determined by intraday precision and interday precision. Intraday precision was determined by preparing six replicates at three different concentrations. An interlaboratory test determined interday precision. The %RSD values were found to be less than 2% [36] (Tables 6 and 7).

Table 6: Intra-Day Precision.

Concentration(µg/ml)	Peak area (cm)			%RSD (%)
	1hr	2hr	3hr	
1	1502.424	1714.445	1587.835	6.66
3	1769.622	1863.782	1803.855	2.62
6	2910.023	3279.425	3200.724	6.21

Table 7: Inter-Day Precision.

Concentration(µg/ml)	Peak area			%RSD (%)
	1 day	2 day	3 day	
1	1627.325	1505.342	1572.889	3.89
3	2026.641	2259.799	2378.858	8.06
6	3653.289	3604.828	3696.582	1.25

Accuracy

An accuracy study was performed at LQC, MQC and HQC levels and % recovery was found to be 98.9%, 98.60% and 98.19 % respectively, which were within the acceptable limits, i.e.98-102% (Table 8). The chromatograms were depicted in Fig. 6, Fig 7, and Fig 8.

Table 8: Accuracy study

Concentration(µg/ml)			Peak area			%Recovery
Formulation	API	Mixture	Formulation	API	Mixture	
1(Low level)	4	5	997.987	3645.4	4591.406	98.9%
3(Medium Level		7	1988.795		6244.885	98.60%
6 (High Level)		10	3606.04		7124.699	98.19%

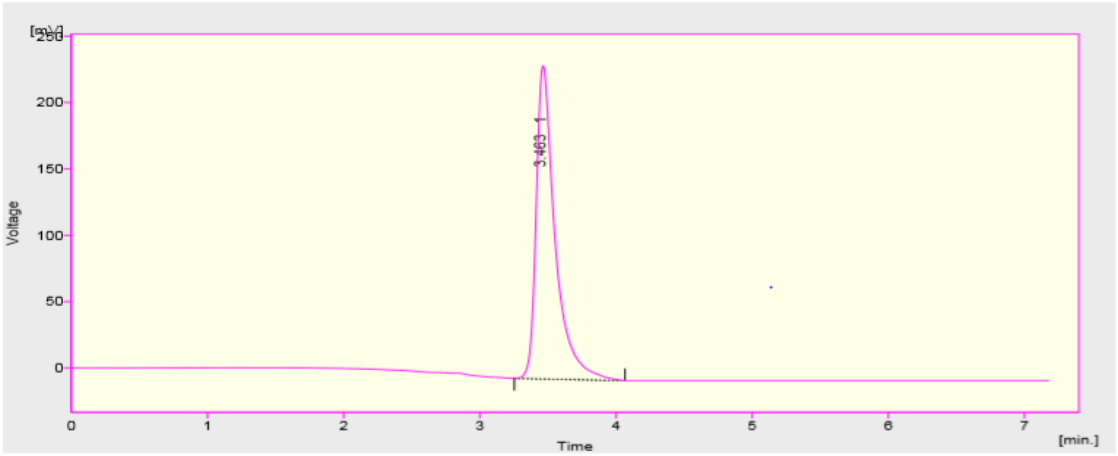

Fig. 6: Chromatogram for accuracy (LQC level).

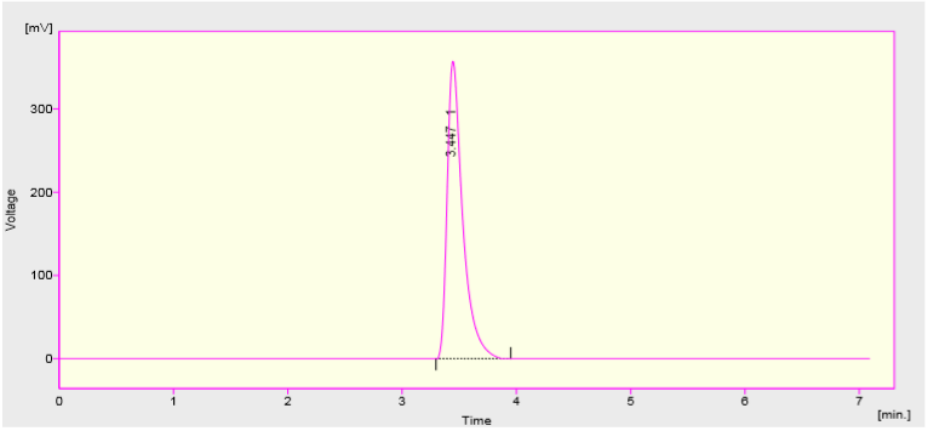

Fig. 7: Chromatogram for accuracy (MQC level).

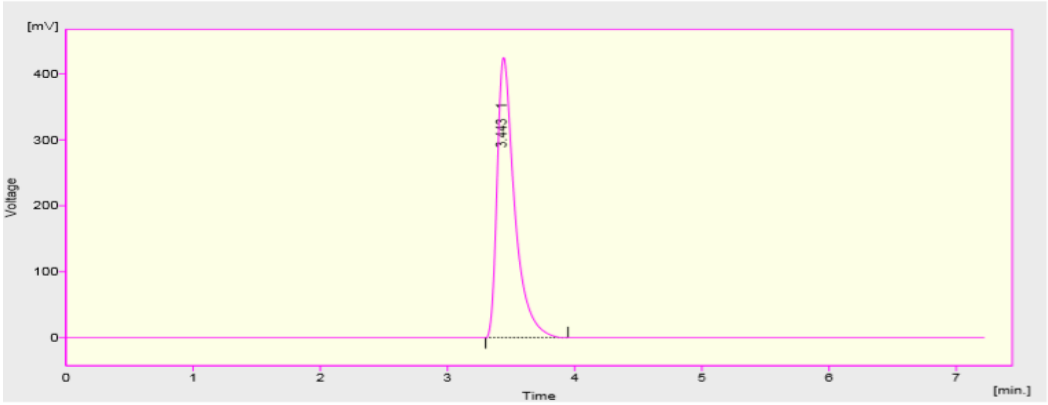

Fig. 8: Chromatogram for accuracy (HQC level).

6.3.7 LOD AND LOQ

LOQ & LOD were determined at a signal-to-noise ratio. The LOD and LOQ were found to be 0.01 and 1 ng/mL.

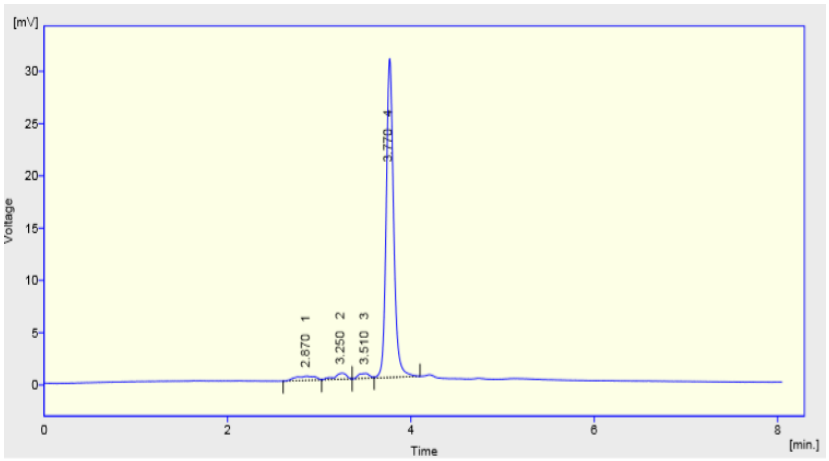

Fig. 9: Representative chromatogram of LOD.

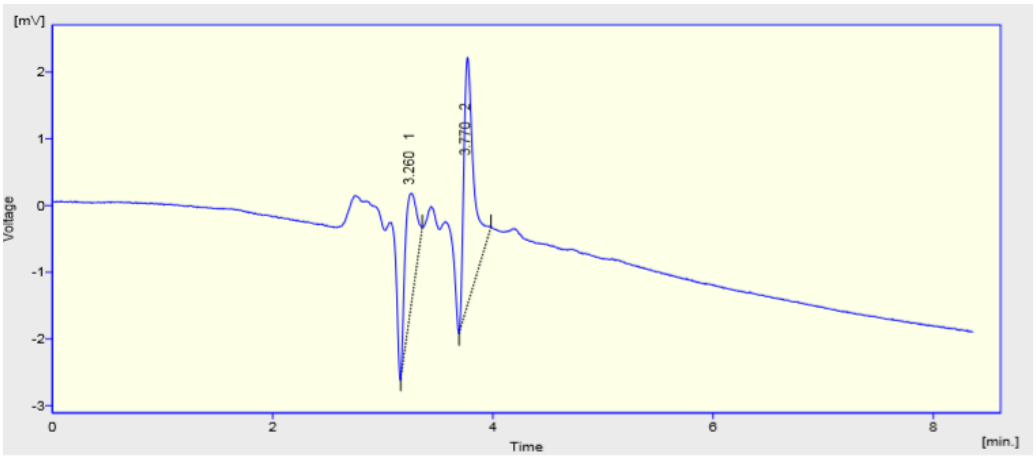

Fig. 10: Representative chromatogram of LOQ

6.3.8 ROBUSTNESS

The robustness of the HPLC method was analyzed by deliberately varying the chromatographic parameters, such as flow rate (± 2 mL/min) and wavelength (± 2 nm). The effect of variation in wavelength and flow rate is depicted in Tables 9 and 10.

Table 9: Effect of variation in wavelength

Concentration (mg/ml)	Wave length(nm)	Mean	Standard deviation	% RSD
3	243	3651.57	45.9	1.26
	245			
	247			

Table 10: Effect of variation in flow rate.

Concentration (mg/ml)	Flow rate	Retention time	Mean	Standard deviation	% RSD
3	0.8	5	1626.01	2501.04	1.92
	1	3.7			
	1.2	3			

CONCLUSION

The procedure established for Erlotinib proved to be simple, accurate, sensitive, fast, rugged, and cost-effective. The conditions established in the analytical procedure with good resolution in a short analysis time. The % RSD for all parameters was found to be within the limit. This shows that the results obtained by this procedure are in good agreement. So the procedure established can be employed for routine analysis of Erlotinib in laboratories and quality control purposes.

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