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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF ERLOTINIB IMPURITIES BY QbD APPROACH

P. A. Kumar [1], Y. R. Kumar [1] and A. Jayashree [2,*]
[1]Analytical Research and Development, IPDO, Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad-500 090, Telangana, India.
[2,*]Centre for Chemical Science and Technology, J. N. T. U, H, Kukatpally, Hyderabad-500072, Telangana, India.
*E-mail: ashokchem2000@gmail.com

ABSTRACT

A novel simple isocratic stability–indicating LC method has been developed for the estimation of impurities in Erlotinib API and formulation products. Chromatographic separation was achieved in an isocratic elution mode by QbD-approach. Quality by Design approach to method development uses statistical design of experiments to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. The present study describes the development of a comprehensive science and risk based HPLC method and subsequent validation for the analysis of Erlotinib drug substances and drug products using a quality by design approach. All the known impurities and unknown impurities were well separated and not interfering with placebo peaks in tablet formulations. The eluted compounds were monitored at 245 nm. Erlotinib was subjected to the stress conditions like oxidative, acid, base, hydrolytic, thermal and photolytic degradation. The degradation products were well resolved from main peak and its impurities, providing the stability- indicating power of the method. The developed method was validated as per international conference on Harmonization guidelines with respect to specificity, limit of detection, limit of quantification, precision, linearity, accuracy, robustness and ruggedness.

Keywords: Erlotinib, Quality by Design approach, Design of Experiments, Degradation Products, Stability-Indicating, ICH Guidelines

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INTRODUCTION

Erlotinib (ERL), chemically known as *N*-(3-ethynylphenyl)-6, 7-bis (2-methoxyetho -xy) quinazolin-4-amine. Erlotinib is an epidermal growth factor receptor inhibitor (EGFR inhibitor) and used to treat non-small cell lung cancer (NSCLC)), the oral epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitor (TKI). Erlotinib is an established second-line treatment for advanced NSCLC.[1- 2] The molecule structure is shown in Figure-1.

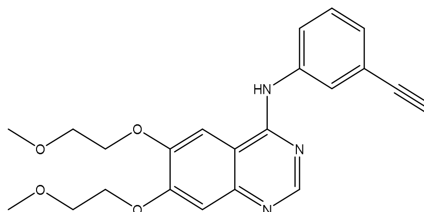

Fig.-1: Chemical structure of Erlotinib

The route of synthesis of ERL and possible degradants resulted, seven known impurities which are not reported in any of the pharmacopeia. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product.[3,4] As per Literature there is no single method available for the

determination of all impurities in a single method.[5-7] It is felt necessary to develop a stability indicating method for ERL related impurities in API and tablet dosage formulation by QbD approach. In the present work the author separated all the degradant impurities from placebo peaks in a simple isocratic RPLC method by QbD approach and validated the method as per ICH guidelines[8]. Method development can be a time-consuming process that can be repeated many times throughout a drug development pipeline. Methods are commonly developed using a one-factor-at-a-time approach where one variable is changed sequentially until a suitable method is produced. This type of development may create an adequate method, but provides limited understandings of method capabilities and method robustness. A better understanding of the overall method capabilities and limitations in development ensures a greater chance of successful method validation, transfer and routine use.

Quality by Design (QbD) is a concept first outlined by well-known quality expert Joseph M. Juran in various publications[9-12], most notably Juran on Quality by Design. While Quality by Design principles has been used to advance product and process quality in every industry, and particularly the automotive industry, they have most recently been adopted by the U.S. Food and Drug Administration (FDA) as a vehicle for the transformation of how drugs are discovered, developed, and commercially manufactured. Since first initiated by the U.S. Food and Drug Administration (FDA) in its "Pharmaceutical cGMPs for the twenty-first century", Quality by Design (QbD) has become an important concept for the pharmaceutical industry that is further defined in the International Conference on Harmonization (ICH) guidance on pharmaceutical development as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified.

The same QbD principles have been applied to the development of analytical methods, and are termed "Analytical QbD" (AQbD) [13]. Analogous to process QbD, the outcome of AQbD is a well understood, fit for purpose, and robust method that consistently delivers the intended performance throughout its lifecycle. The broad knowledge obtained from this process is used to establish a method operable design region (MODR), a multidimensional space based on the method factors and settings that provide suitable method performance. It is also used to establish meaningful method controls of which system suitability is one component.

EXPERIMENTAL

Materials and Methods

ERL tablets, standards of ERL and its seven impurities namely 3-ethynylaniline; 6,7- bis- (2-methoxyethoxy)-4-quinazolinone; 6,7-bis-(2-methoxyethoxy)-4-isopropoxyqui nazo-line; 4-chloro-6,7-bis-(2-methoxyetho xy) quinazoline; [6,7-bis-(2-methoxy-ethoxy) -quinazolin-4-yl]-(3-bromo-phenyl)-amine hydrochloride; Erlotinib Dimer impurity and ERL III impurity were supplied by Sicor de mexico s.a de c.v. limited. Tetrahydrofuran, trifluoroacetic acid and acetonitrile were purchased from Merck, India. High purity water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA). The purity of all chemicals was above 98%.

Equipment

The Waters HPLC system (Waters, UK) used consists of a pump, auto sampler and a PDA detector. The output signal was monitored and processed using empower-3 software. Cintex digital water bath was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India).

Chromatographic conditions

The method was developed using Waters, X bridge C18, 150 x 4.6 mm, 3.5µm column with mobile phase containing a mixture of water, acetonitrile, tetrahydrofuran and trifluoroacetic acid in the ratio of 900:30:70:1.5, v/v/v/v respectively. The HPLC system operated with an isocratic elution mode at flow rate of 1.5 mL/min and the column oven temperature was maintained at 50°C. The injection volume was

20 µl. UV detection was carried out at 245 nm and data acquisition time of 60 min. Diluent was prepared by mixing in the ration of Acetonitrile: Water (50:50 v/v)

Preparation of Standard Solution

About 27 mg of Erlotinib HCl into a 50 mL of volumetric flask, sonicated to dissolve completely and filled to volume with diluent. Further diluted 5 mL of this solution to 25 mL with diluent. Pipetted 1 mL of above solution into a 100 mL volumetric flask, this standard solution contains 0.001 mg/mL of Erlotinib.

Preparation of Sample Solution

Transferred an accurately weighed portion of the tablets powder, equivalent to about 100 mg of Erlotinib into a 100 mL volumetric flask to this added 70 mL of diluent and sonicated for 15 minutes with occasional shaking and filled to volume. Filtered the solution through 0.45µ GHP membrane filter. Pipetted 5 mL of this solution into a 10 mL volumetric flask and filled to volume.

Experimental Design

The experimental design (regular two level factorial), desirability function and statistical data analysis calculations were performed by using Design-Expert® version 9.0.6 (Stat Ease Stat-Ease, Inc., Minneapolis, MN, USA).

Several types of experimental designs (e.g. two levels full factorial, two level fractional factorial, Placket-Burman, mixed level designs) are available and these designs allow the simultaneous examination of qualitative, quantitative and mixture related factors.

RESULTS AND DISCUSSION

Initial method development

The main objective of the chromatographic method was to separate all the impurities of ERL, from each other and from the placebo peaks. A blend solution prepared from the tablets containing 500 µg/mL of ERL and spiked with 1 µg/mL (0.20%) of each impurity dissolved in diluent and used for method development. A placebo solution was prepared as per test preparation and used to identify the placebo peaks. Before starting the development impurity mix, placebo and degradation samples analyzed with different HPLC method, it was observed that base line was not good. To achieve shorter run time and good baseline different organic solvents along with different compositions in different columns were tried for the separation.

The chromatographic separation was achieved on X Bridge C18, 150 x 4.6mm, 3.5 µm column with mobile phase containing a mixture of water, acetonitrile, tetrahydrofuran and trifluoroacetic acid in the ratio of 900:30:70:1.5 v/v/v/v respectively. Flow rate was 1.5 mL/min and the column oven temperature was maintained at 50°C. The injection volume was 20 µl and UV detection was carried out at 245 nm. After this initial optimization, method was subjected to factorial design to study the variables which can influence the resolution between the impurities.

Method Optimization by Design of Experiments

A two level full factorial design was selected for the present study to determine the main effects and all interactions between the factors, leading 2^f experiments, where f is factors. During the preliminary study, factors (f) which could have significant affects were extracted for further analysis. Based on the initial separation flow rate, Acetonitrile ratio, THF ratio and column temperature were selected as critical parameters (Table-1) to evaluate the quality target method profiles (resolution and RRT) and critical quality attributes. Evaluating all of these parameters with a full factorial design would involve $2^4 = 16$ trials. Total 16 runs were performed. In all the experiments R_S^1 (Resolution between impurity 6 & ERL), R_S^2 (Resolution between impurity 5 & ERL) and Rs^3 (RRT of Impurity 7) were monitored. These experiments were performed and the results are summarized in the (Table-2)

Table-1: Factors and Critical Quality Attributes

CMPs	Range of Each Parameter Used for DOE			QTMP	CQA
	Original Condition	Low Level	High Level		
% ACN in MP	30%	27%	33%	Resolution NLT 2 RRT of Imp 7	Rs1 : between ERL & Imp 6 Rs2: between Imp 5 & ERL Rs3: RRT of Imp 7
%THF in MP	70%	63%	77%		
Column Temp.	50°C	45°C	55°C		
Flow rate mL/min	1.5	1.4	1.6		

RRT: Relative retention time; Critical Method Parameters (CMPs); Quality Target Method Profile (QTMP); Critical Quality Attributes (CQA)

Table-2: Matrix of Experiments for 2 Factorial Designs

	Factor 1	Factor 2	Factor 3	Factor 4
Run	A: % ACN	B: % THF	C:Flow rate mL/min	D: Col.Temp. °C
0*	30	70	1.5	50
1	27	77	1.6	55
2	33	63	1.4	55
3	27	63	1.4	55
4	33	63	1.4	45
5	33	77	1.6	55
6	27	63	1.6	55
7	27	77	1.4	45
8	27	77	1.4	55
9	27	63	1.4	45
10	33	63	1.6	55
11	33	63	1.6	45
12	27	63	1.6	45
13	27	77	1.6	45
14	33	77	1.4	45
15	33	77	1.6	45
16	33	77	1.4	55

*actual conditions

The results (Table-3) after completion of the 16 experiments were analyzed through Design Expert ®software. The effect on the three dependent variables with the independent variables was explained by using Cubical graphs (Figures-2, 3 and 4).

Table-3: Response factors Values in 2 Factorial Design

Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
	A: ACN	B:THF	C:Flow rate	D: Col. Temp.	Resolution 1	Resolution 2	RRT
0	30%	70%	1.5mL/min	50°C	ERL & Imp 6	Imp 5 & ERL	Imp 7
1	27	77	1.6	55	4.3	2.7	1.83
2	33	63	1.4	55	2.8	4.4	1.86
3	27	63	1.4	55	0.2	5.2	1.89
4	33	63	1.4	45	7.6	4.3	2.15
5	33	77	1.6	55	1.1	0.2	1.66
6	27	63	1.6	55	0	2.4	1.89
7	27	77	1.4	45	10.3	2.4	1.86
8	27	77	1.4	55	4.6	2.7	1.83
9	27	63	1.4	45	3.5	2.4	2.45
10	33	63	1.6	55	2.4	4	1.86

11	33	63	1.6	45	7.3	4.4	1.89
12	27	63	1.6	45	3.9	3.5	1.98
13	27	77	1.6	45	9.6	2.4	1.87
14	33	77	1.4	45	2.6	0.2	1.88
15	33	77	1.6	45	3.8	0.1	1.85
16	33	77	1.4	55	1.5	0.2	1.76

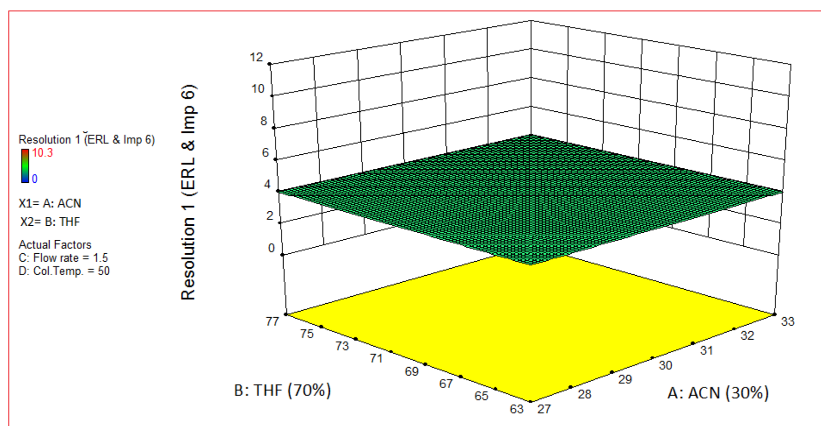

Fig.-2: 3D Surface Model Graph for Response 1 (Resolution 1)

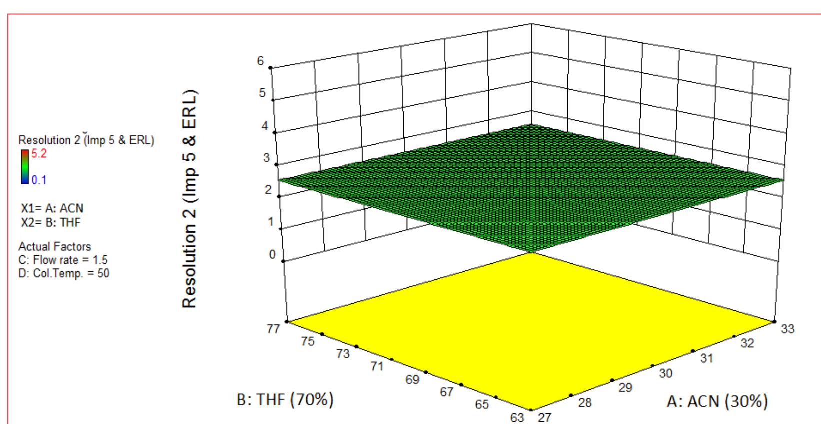

Fig.-3: 3D Surface Model Graph for Response 2 (Resolution 2)

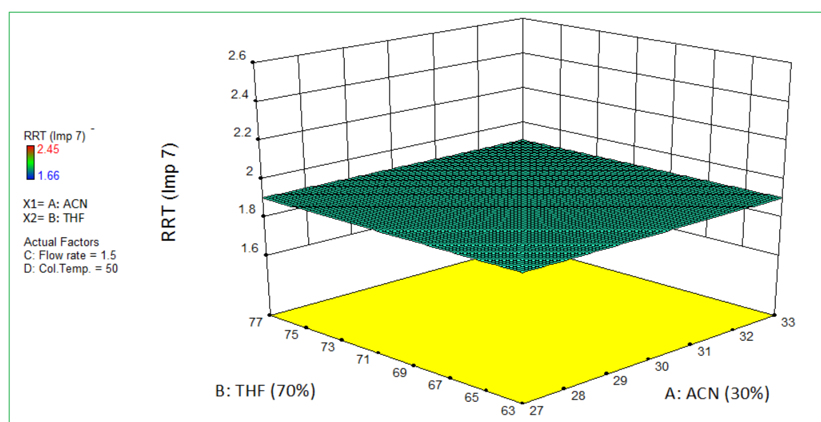

Fig.-4:3D Surface Model Graph for Response 3 (RRT of Imp 7)

Significant affects were observed due to the column temperature (°C), acetonitrile ratio and THF ratio in the mobile phase. No significant affect was observed due to the flow rate of the mobile phase. The desirability zones for Resolution 1 (between ERL & Imp 6) and Resolution 2 (between Imp 5 & ERL) and RRT of Impurity 7 were maximal towards blue (yellow) to red as a function of constant flow rate i.e., 1.5 mL/min, and constant column temperature i.e., 50°C. And the optimized conditions are shown in green zones. Based on Design Expert analysis, the desirability/3D graphs (Figures-5, 6 and 7) indicated that the maximum desirability was achieved for (a) amount of acetonitrile in mobile phase is about 28.77%, (b) amount of THF in mobile phase is about 70%, (c) column temperature is about 48.8°C, and (d) flow rate is about 1.5 mL/min. The predicted values of Response (95% CI low & 95% CI high), i.e., Resolution 1 is 4.09 (2.43 & 5.76), Resolution 2 is 2.59 (1.70 & 3.49), and RRT is 1.91 (1.81 & 2.00) were obtained from numerical optimization and point prediction calculations of post analysis (Figure- 2, 3and 4).

To confirm the point prediction values, experiments (n = 2) were conducted to determine the mean responses of Resolution 1, Resolution 2 and RRT and found to be similar as predicted values.

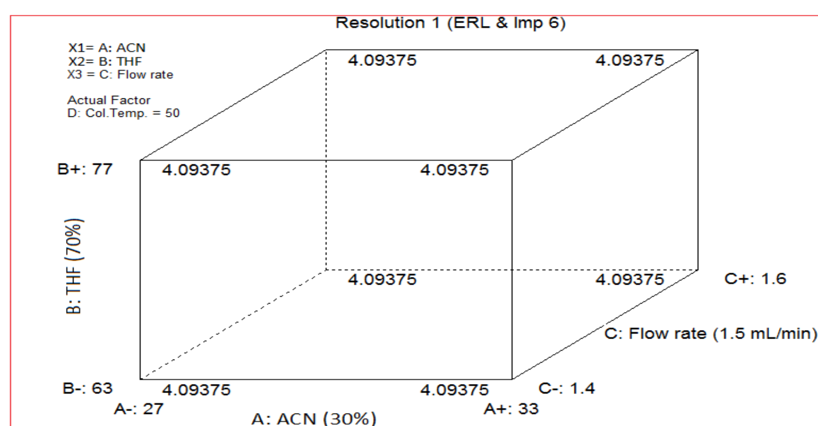

Fig.-5: 2 Factorial Design Cube Graph for Desirability of Response 1

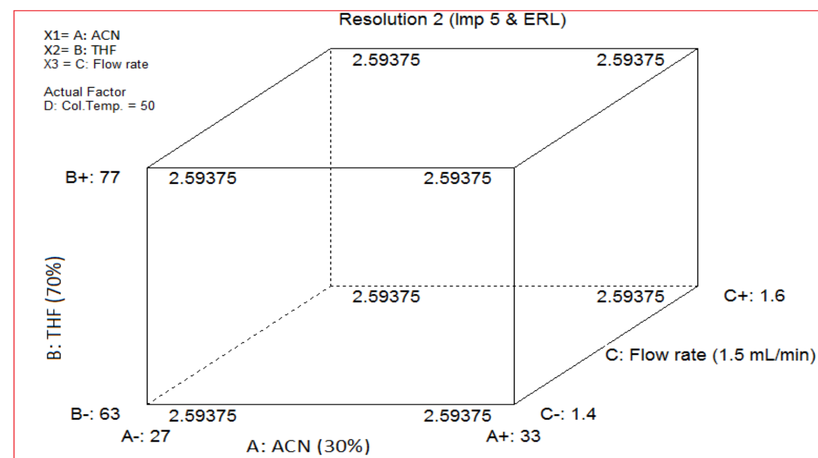

Fig.-6: 2 Factorial Design Cube Graph for Desirability of Response 2

From the pareto chart the resolution between Erlotinib and Impurity 6 (R_S^1) was majorly affected by organic phase Acetonitrile ratio, THF ratio and followed by mixed interaction of column temperature and Acetonitrile ratio, THF ratio. The resolution between impurity 5 & ERL was affected by mixed interaction of Acetonitrile ratio, THF ratio and column temperature. The RRT of impurity 7 was affected by Flow rate, Organic phase composition followed by mixed interactions. The definition for design space

of a LC method can be “multidimensional combination and interaction of mobile phase variables (Acetonitrile, THF) and chromatographic parameters (column temperature) that have been demonstrated to provide assurance of result obtained with the method”. The yellow region in Design space graph indicates the responses are in acceptable range and the grey region shows the responses are below the desired level. The initial method development parameters were lying in middle of the design space; hence the initial developed method was finalized and performed method validation.
The overlay chromatogram of placebo and spiked sample was shown in Figure-8 representing no interference of placebo peaks with the known and unknown impurities of ERL. Also it clearly shows excellent separation between each pair of compounds.

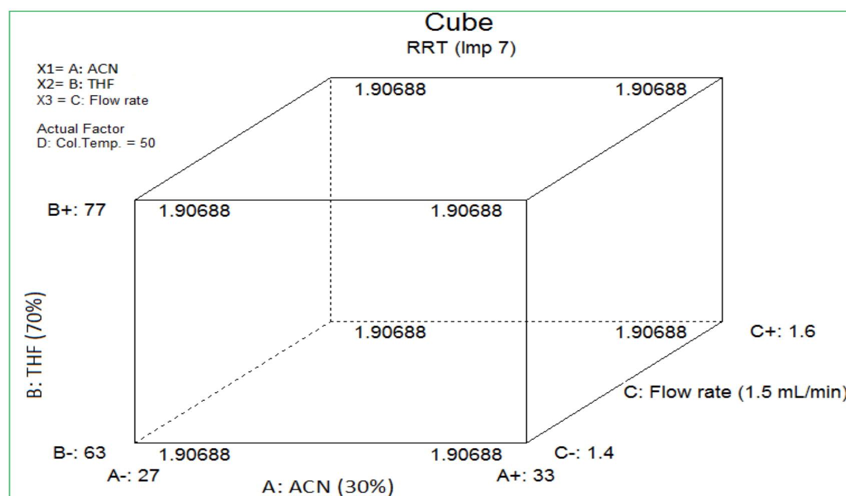

Fig.-7:2 Factorial Design Cube Graph for Desirability of Response 1

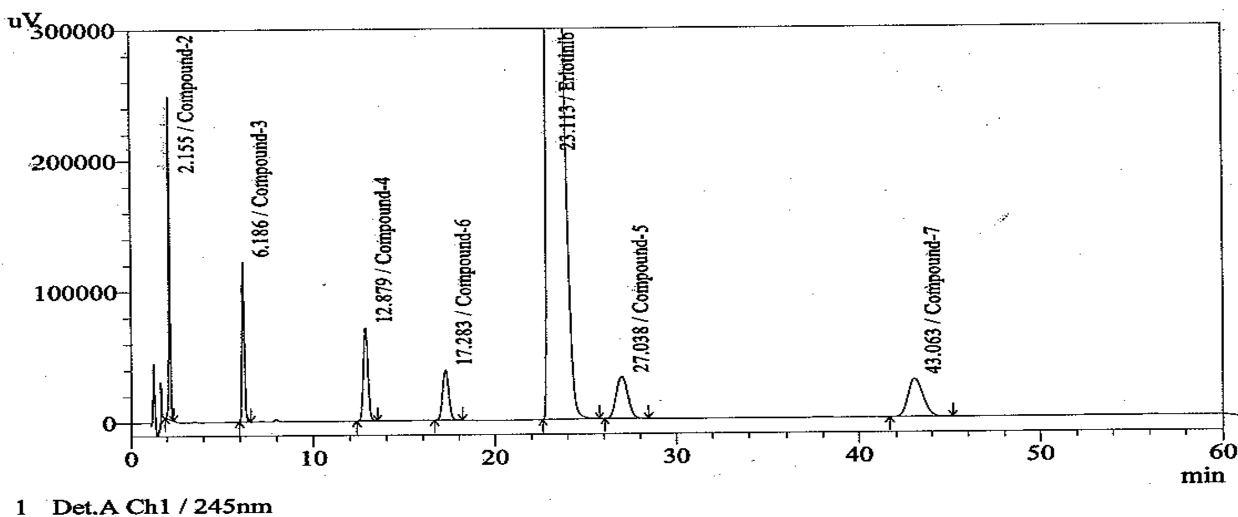

Fig.-8: All impurities spiked Chromatogram

Method Validation

As part of method validation Specificity, Precision, Linearity, LOD-LOQ, Accuracy, Robustness and Solution stability, parameters are verified.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the LC method for ERL was carried out in the presence of its impurities

namely impurity 2, 3, 4, 5, 6 and 7. Stress studies were performed for ERL API and tablets to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted with a stress condition of Photolytic (1.2 Million Lux hours followed by 200 Watt hours), thermal (70°C, 6hour), acid (1N HCl, 6hours on bench top), base (1N NaOH, 6hours on bench top) and oxidation (10% H_2O_2, 6hours on bench top) to evaluate the ability of the proposed method to separate ERL from its degradation products. Peak purity for the ERL peak was evaluated by using PDA detector in all stressed samples. Erlotinib hydrochloride was found to be stable during the Photolytic, Acid, Alkaline, Oxidation, UV & Thermal treatments of raw material and finished product preparations.

Precision

The precision of the method verified by repeatability by injecting six individual preparations of ERL API and tablets spiked with 0.20% of its impurities (0.20% of impurities with respect to 0.5 mg/mL of ERL). % RSD of area for each impurity was calculated. The same experiment was also evaluated using different instrument and performing the analysis on different day. The results of precision and intermediate precision were well within the limits of %RSD.

Limits of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ for impurities 2, 3, 4, 5, 6 and 7 were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at LOQ level by injecting six individual preparations of impurities and % RSD was calculated. The LOQ levels for active as well as for all the impurities were found to be below 0.05% with respect to test concentration.

Linearity

Linearity test solutions for the method were prepared by diluting stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% of the specification level. The Squared coefficient of correlation for all the peaks was found to be more than 0.999.

Accuracy

Accuracy of the method was evaluated in triplicates using concentration levels LOQ, 25%, 50%, 100% and 200% on ERL tablets. Standard addition and recovery experiments were conducted on real sample to determine accuracy of the related substance method. The percentage of recoveries for all ERL impurities and ERL were calculated and the recoveries were within the range (95%-105%), % Related standard deviations are below 3.0.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between ERL and its impurities and tailing factor for ERL were recorded. The flow rate of the mobile phase was 1.5 mL/min, to study the effect of flow rate on the resolution; flow was changed by 0.2 units from 1.3 to 1.7 mL/min. The effect of the column temperature on resolution was studied at 45°C and 55°C instead of 50°C.

Solution stability

Solution stability of ERL and its impurities in the related substances method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks for 72hours at room temperature. Content of impurities 1, 2, 3, 4, 5, 6 and 7 were determined for 72 hours.

CONCLUSIONS

The simple isocratic reverse phase LC method was developed by QbD approach for quantitative analysis of ERL and its impurities in ERL drug substances and drug products. The method is validated as per ICH

guidelines and found to be specific, precise, linear, accurate, rugged, and robust. Satisfactory results were obtained from validation of the method. The method was stability-indicating and can be used for routine analysis of production samples and to check the stability of samples of ERL formulations.

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