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Development and Validation of a Stability Indicating HPLC Method for Determination of Erlotinib Hydrochloride in Bulk

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Abstract : Determination of Erlotinib hydrochloride in the presence of its degradation products was studied and validated by a RP-HPLC method. The RP-HPLC method was developed for the chromatographic separation of Erlotinib and its impurities by using Hibar C_{18} (250x4.6 mm, 5 µm) column with a mobile phase combination of 10 mM ammonium formate with pH-4.0 and acetonitrile in isocratic elusion with an injection volume of 20 µl and flow rate was 1.0 ml/min and detection was carried a wavelength of 290 nm. Further, stress studies for acidic, basic, neutral, oxidative, and thermal degradations studies were carried out as per ICH guidelines An MS/MS study has been performed on the degradation products to predict the degradation of Erlotinib. The method provided linear responses over the concentration range of 100–1500 ng/ml and regression analysis showed a correlation coefficient value (r^2) of 0.995. The LOD and LOQ were found to be 1 ng/ml and 3 ng/ml, respectively. The developed LC method was validated as per ICH guidelines with respect to accuracy, selectivity, precision, linearity, and robustness.
Key words : Erlotinib, Degradation, RP-HPLC, ICH.

Introduction

Force degradation studies are also known as stress studies, stress decomposition studies, stress testing and so on. Forced degradation is a process that involves degradation of drug products and drug substances. Stability testing aims to prove the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors (temperature, humidity where appropriate, oxidation, photolysis, and susceptibility to hydrolysis across a wide ranges of pH values)

The ICH guideline states that stress testing is to identify the likely degradation products and to determine the intrinsic stability of the molecule and establishing degradation product, and to validate the stability indicating procedures.

The presence of impurities can have a significant impact on the product quality, safety and efficacy, hence the percentage level of impurities need to control in the drug substance as well as a drug product. An extensive literature survey reveals that there were few analytical methods available for the determination of Erlotinib in plasma, bulk and in pharmaceutical dosage forms by liquid chromatography and liquid chromatography mass spectrometry (LC-MS) techniques [1-6]. The objective of the current work is to develop a simple, precise, accurate, robust and stability indicating LC-MS/MS compatible HPLC method for the

determination of Erlotinib hydrochloride and its related impurities in bulk, followed by method validation as per current International conference on harmonization (ICH) guidelines.

Materials and Methods

Chemicals and Reagents:

Working Standard of Erlotinib was obtained from the manufacturers as gift sample. Acetonitrile of HPLC and LC-MS/MS grade, Formic acid AR grade and Ammonium Formate AR grade, were supplied by rankem Chemicals. Water HPLC grade was obtained from Milli-Q RO system (Millipore, Bedford, USA) was used for buffer and sample preparation.

Equipment & Chromatographic condition:

High Performance Liquid Chromatography **(**Shimadzu gradient HPLC system**)** equipped with a solvent delivery system **(**Model-LC-10 AT-VP**)**, Rheodyne injector **(**Model-7725i with 20µl loop**)**, UV detector **(**Model-SPD M-10A VP). The data were recorded using Class VP data station software. Hibar C_{18} (250x4.6 mm, 5 µm) column was used for method development and validation at ambient temperature **(**25°C**).**In this current method the separation was achieved using 1ml/minflow rate, detection at 290nm with an injection volume of 20µl.

Stress Degradation Studies:

The stress degradation study on Erlotinib was performed as per ICH guideline Q1A (R2)[7]. For each forced degradation condition, the sample was dissolved in 25 ml of water and degradation samples were prepared with acid, alkali, hydrogen peroxide, and water to get a concentration of 1000 µg/ml and it was subjected to different stress conditions with each 25 ml of 0.1 N HCl, 0.1 N NaOH, 3% H_2O_2, and water at 60ºC.

Preparation of Sample Solutions:

Samples of acid and base hydrolysis were neutralized by 0.1 N NaOH and 0.1 N HCl, respectively. The solutions of acid, base, thermal, neutral, and peroxide degradation were diluted with mobile phase to obtain a concentration of 100 µg/ml before injecting into the system.

Validation:

The proposed method was validated as per ICH guidelines[8]. Standard solution of Erlotinib (1 mg/ml) was diluted to obtain solutions in the concentration range of 100–1500 ng/ml to ascertain the linearity and range. The solutions were injected in triplicate of about 20 µl. The calibration curve was plotted against the peak areas and corresponding concentrations. Intraday and interday precisions were carried out by analyzing three different concentrations levels 250, 500, and 750 ng/ml in triplicate. Accuracy was determined in terms of recovery by analyzing QC levels 250, 500, and 750 ng/ml spiked with the degradation sample in triplicate and calculating the percent recovery. The S/N ratio method was used to determine the detection and quantification limits. The robustness of the method was determined by minor deliberate changes in the chromatographic conditions.

Results

Method Development:

Various solvent systems (acetonitrile, water, methanol) and buffer mixtures in different ratios, having different pH ranges (2.5–8) and flow rates (0.8–1.2 ml/min), were evaluated. The best separation of the drug and degradation products were achieved by using 10mM ammonium formate buffer (pH 4.0) and acetonitrile in the ratio of 62:38 v/v as the mobile phase and the Hibar C_{18} (250x4.6 mm, 5 µm) column as the stationary phase. The analyses were carried out in isocratic elution mode using a flow rate of 1.0 ml/min, injection volume of 20 µl at room temperature, and the detection of an analyte was recorded at 290 nm. The mobile phase was

filtered through a 0.22 µm polytetrafluoroethylene membrane filter before using it as a mobilephase for HPLC system and the chromatograms were recorded using Class VP software.

Validation:

The developed stability-indicating method was validated as per ICH guidelines in terms of linearity, precision, accuracy, specificity, detection, and quantification limits. The detector response for the drug was found to be linear over the concentration range of 100-1500 ng/ml (r^2 =0.994; n=6). Accuracy was calculated in terms of percent recovery upon spiking a combination of stressed samples with the three known concentrations of the real samples, viz., 250, 500, and 750 ng/ml and the results were found to be between 99.46%- 100.17% (**Table 1**). Precision results (intraday and interday) are shown in **Table 1**. The % RSD values for the intra- and interprecision studies were found to be less than 1.9 and 1.4, respectively, which confirmed that the developed method was adequately precise. Detection and quantification limits were found to be 1 ng/ml and 3 ng/ml, respectively which indicate the method is more sensitive than the reported methods.

Table 1. Recovery, Precision studies for the Erlotinib hydrochloride

Actual Conc. (ng/ml)	Recovered Conc (ng/ml) ±SD; RSD% (n=6)	% Recovery	Intraday calculated concentration (ng/mL) ± S.D.; RSD % (n=5)	Interday calculated concentration(ng/mL) ± S.D.; RSD % (n=5)
250	245.66±3.785;1.532	99.46	250.23 ± 2.081; 0.831	248.26 ± 3.311; 1.324
500	498.66±3.055;0.621	99.93	494.56± 9.073; 1.830	497.23 ± 6.306; 1.256
750	751.23±2.516;0.313	100.17	747.36 ± 2.081; 0.278	747.23 ± 5.537; 0.731

Discussion

Stress Degradation:

The degradation of Erlotinib under various forced degradation conditions like acid, base, neutral, oxidation, thermal, and photolytic under UV light were examined by liquid chromatography and the chromatograms were recorded. The typical HPLC chromatogram and mass scan spectra of the drug products are depicted in **Fig 1 and 2**, respectively. The degradation behavior of Erlotinib under various stress conditions is shown in **(Table 2).**Acid, Base, and Neutral Hydrolysis.In base hydrolysis, degradation more than 30% was observed after 24 hr. However, 10% degradation was observed in the case of acid hydrolysis and 4% on neutral degradation. The drug was tested under 3% hydrogen peroxide for 24 hr and the degradation was greater than 90%**(Fig 3).**

Table 2. Results of stress degradation studies of Erlotinib hydrochloride

S.No	Time (hrs)	Basic hydrolysis (% degradation) 0.1N NaOH	Acid hydrolysis (% degradation) 0.1N HCL	Neutral degradation (% degradation) H20	Oxidative degradation (% degradation) 3% H202	Photo degradation (% degradation) UV
1	0	0	0	0	0	0
2	2	1.94	1.26	1.27	8.29	1.14
3	4	5.22	4.39	2.08	15.48	1.89
4	6	12.35	5.18	3.10	33.96	2.20
5	8	24.19	7.93	3.56	50.74	3.15
6	12	30.11	8.75	4.23	79.39	3.98
7	24	32.98	10.22	4.99	90.39	6.36

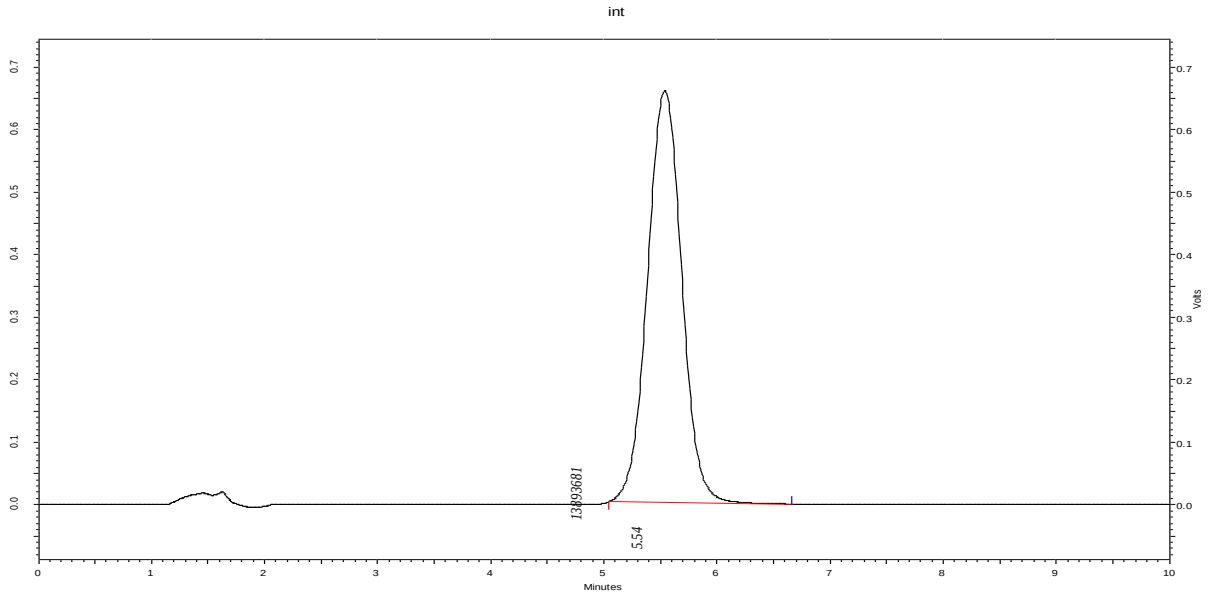

Fig 1. Typical HPLC chromatogram of standard Erlotinib hydrochloride

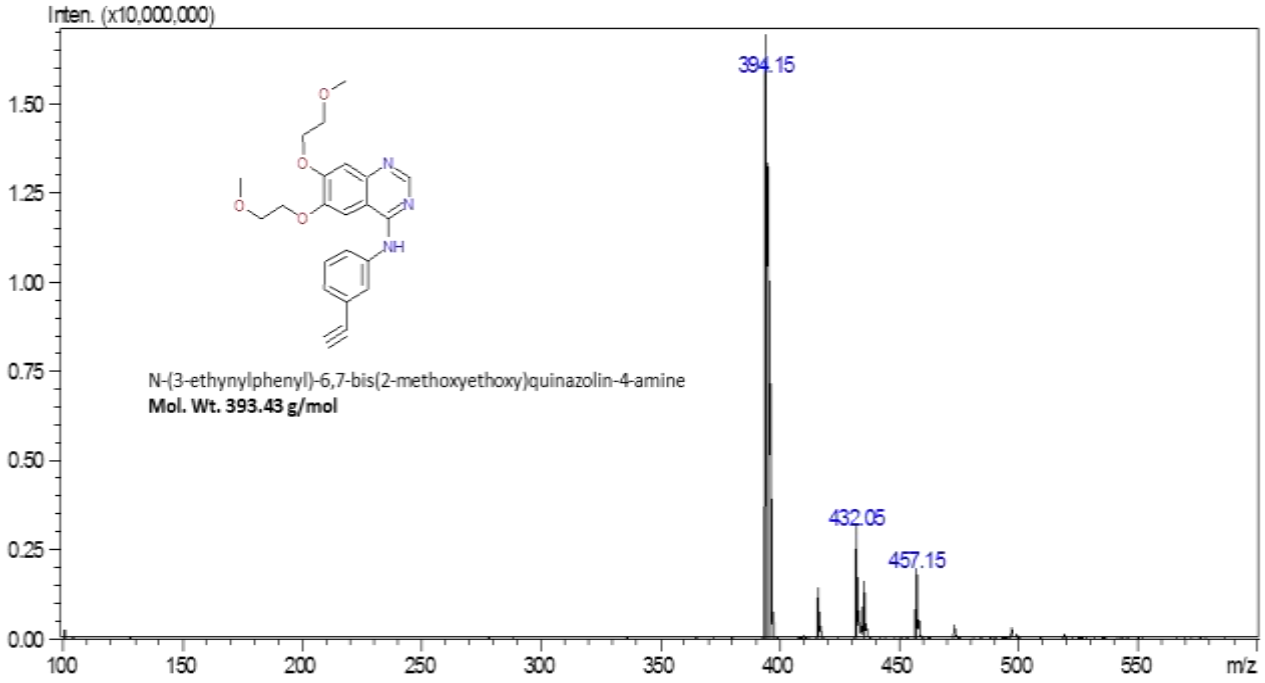

Fig 2. Typical Mass scan spectra of standard Erlotinib hydrochloride

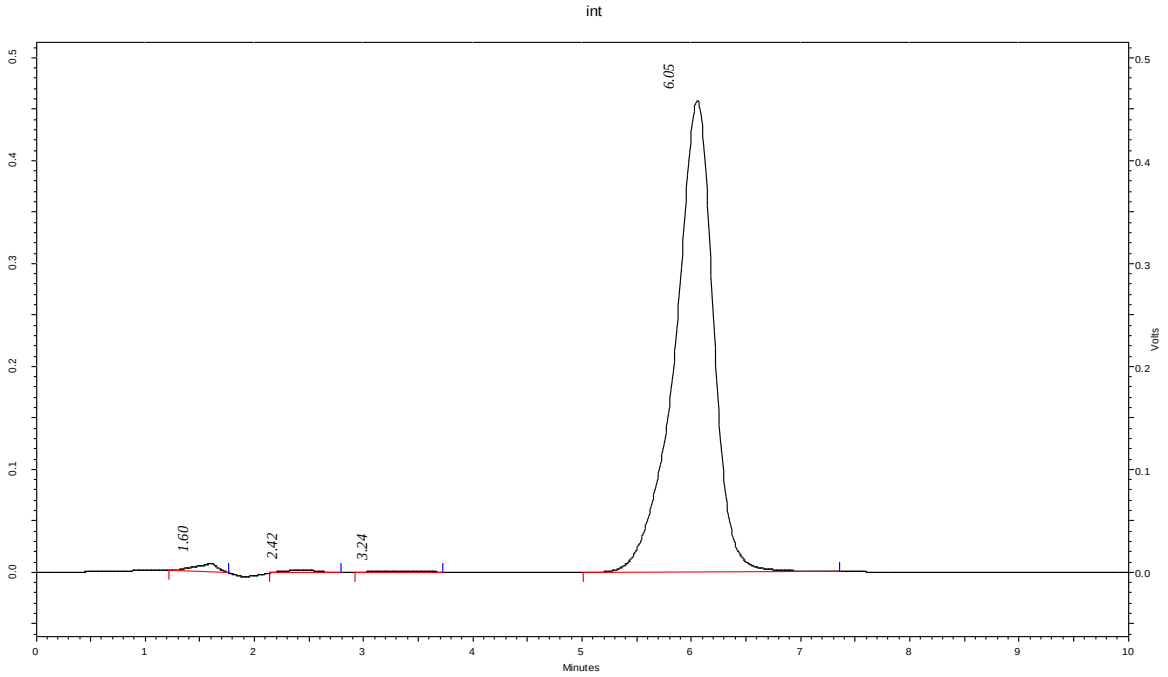

Fig 3. Typical chromatogram showing degradation of Erlotinib hydrochloride

Photolytic Degradation:

The solution of Erlotinib 100 µg/ml was irradiated under UV light at 245 nm and 365 nm for 24 hr and the chromatograms were recorded, which depicted that 6% of the drug has been degraded.

MS/MS Studies:

The samples (10 µL) were injected directly into the source by a flow injection method using water and acetonitrile in the ratio 20: 80 v/v as the mobile phase at a flow rate of 0.5 mL/min, using nitrogen as the drying gas. The forced degradation samples were analyzed on LC-MS/MS to obtain the molecular masses of the potential degradant. The typical ion source conditions were: nebulizer gas, 60 psi; dry temperature, 350°C; dry gas, 5.0 mL/min; capillary voltage, 5kV; vaporizer temperature, 400°C; and dwell time, 200 ms. In ESI positive ion mode, the mass spectrum of the drug degradant has shown molecular ion peaks at m/z values of 186 and 249, respectively **(Figure 4).**

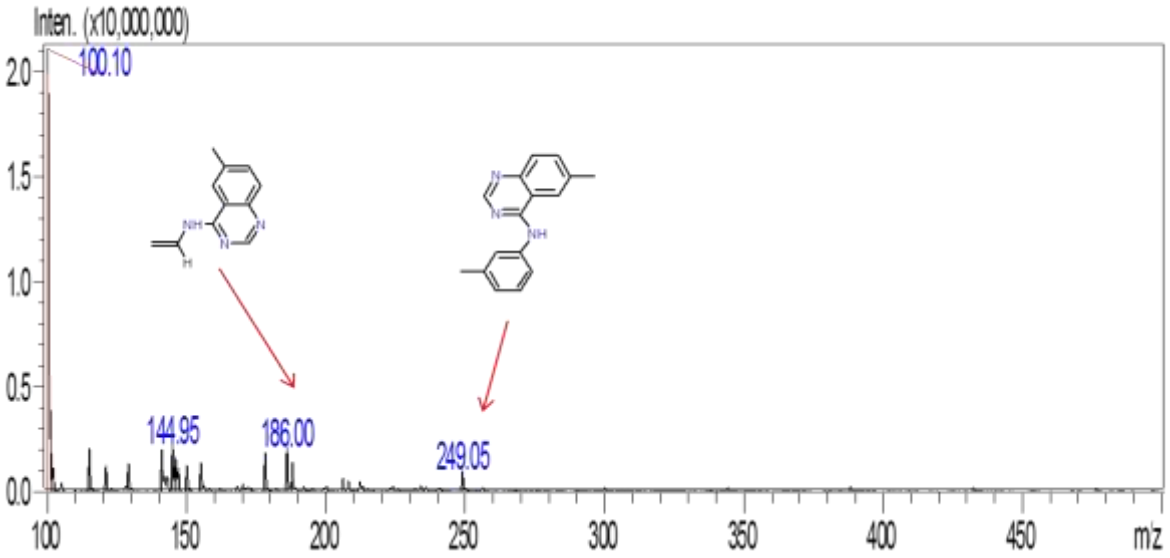

Figure 4. Mass spectra of degradation products from Erlotinib hydrochloride

Conclusion

A stability-indicating liquid chromatography method has been developed using a UV detector. An attempt was made to explore the degradation behavior of Erlotinib by exposing it to ICH-defined stress degradation conditions. The developed method was able to separate Erlotinib from its degradation products. The drug was found to be slightly degraded under acid, neutral, hydrolysis and photolysis conditions. Degradation product was formed under oxidative degradation and base hydrolysis. Thus, the developed method can also be used for the identification of stress degradation products along with routine quality control analysis.

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