

# ***In vitro* drug-drug interaction study of Erlotinib hydrochloride with proton pump inhibitors in aqueous medium and rat plasma using RP-HPLC**

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## **Abstract**

In the present work, *in vitro* drug-drug interaction of anticancer drug Erlotinib hydrochloride with proton pump inhibitors (PPIs) was studied by using RP-HPLC. The method was developed on C<sub>18</sub> column maintained the constant temperature and wavelength at 254 nm. The selected mobile phase was acetonitrile (A) and phosphate buffer (B) (50:50 v/v). The isocratic mode was employed at flow rate 1 ml/min and injection volume was 20 µl. Drug-drug interaction was performed for Erlotinib hydrochloride with three different PPI at pH 1.4, 2.4 and 4.5 in aqueous medium and in plasma. After performing *in vitro* drug-drug interaction study at pH 1.4, pH 2.4 and pH 4.5, the observed result is no change in retention time of Erlotinib hydrochloride in different pH and in combination with PPI in aqueous medium and in rat plasma. From the obtained results, it can be concluded that Erlotinib hydrochloride neither interacted nor formed any stable complex with PPI.

**Keywords:** Erlotinib hydrochloride, Proton pump inhibitors, drug-drug interactions.

## **Introduction**

Erlotinib (N-(3-ethynylphenyl)-6,7-bis (2-methoxy ethoxy)-4-quinazolinamine) is a oral drug used for the treatment of cancer. It is used to treat non-small cell lung cancer that has spread to other parts of the body in patients who have already been treated with at least one or other chemotherapy. Erlotinib hydrochloride has recently been shown that is potent inhibitor of JAK2V617F (mutant of tyrosine kinase JAK2) activity. JAK2V617F is found in most patients with polycythemia vere (PV) and a substantial proportion of patients with idiopathic myelofibrosis or essential thrombocythemia. The study suggests that Erlotinib may be used for treatment of JAK2V617F-Positive PV and other myeloproliferative disorders (Chakravarthy K et al., 2011).

Nowadays multiple drug therapy is a common and useful practice for the treatment of diseases where two or more drugs are given at the same time or concurrently. The drugs may exhibit effects independently or may interfere or interact with each other. The interaction may be potentiation or antagonism of one drug by another. Sometimes multiple drug therapy is beneficial to the patients and sometime it causes serious harmful effects. Thus the drug interaction study is very much important in respect to both bio-pharmaceutics and pharmacology (Shuvashis S et al., 2013). However, to take any step to manage the interaction problems, the nature of interaction should be known. We should know the possible interaction of a new drug prior to use clinically. For the drugs which are being used conventionally, interaction studies are also very important to detect the problems yet to be found out.

High performance liquid chromatography is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture. Retention time and absorbance peak of one species in solution may be changed due to the interaction with other species (David Harvey., 1997). In the present study, analysis of a combination of Erlotinib hydrochloride and proton pump inhibitors (1:1 molar ratio) was carried out using RP-HPLC at pH 1.4, 2.4 and 4.5.

## **Research Methodology**

### **Chemicals and reagents**

Erlotinib hydrochloride was purchased from Indian Fine Chemicals from Mumbai. Proton pump inhibitors like Pantaprazole, Rabeprazole and Omeprazole are purchased from Yarrow Chemical Products. Methanol (HPLC grade) was purchased from Rankem, Mumbai. Triethylamine (TEA, HPLC grade), Millipore water was obtained from Millipore system (Aquelix-5) from our laboratory. All other chemicals used in the analysis were of AR grade.

### **Instrumentation**

Shimadzu HPLC model SPD-M20A was used for method development and validation. PDA detector is used. It is a multichannel detector contains an ideal sensor for an entire spectrum in a uv/vis dispersive spectrophotometer. These are useful in both research and quality assurance laboratories and provides users most advanced level of sensitivity. Eclipse plus C<sub>18</sub> columns are designed for superior peak with basic compounds and deliver high efficiency and excellent peak shape with all sample types. Eclipse plus C<sub>18</sub> is especially useful for the separation of acidic, basic and other highly polar compounds by reverse-phase liquid chromatography (250 × 4.6 mm, particle size is 5 μm). The binary mobile phase consisted of a mixture of A and B which was filtered through a membrane filter 4.5 μm. The solvents were degassed before running at a flow rate of 1 ml/min. The column temperature was ambient at 30 °C. The 20 μl volume of sample was injected and peaks were detected at 240 nm.

## Selection of analytical column

Selection of column plays an important role in the method development. For most of the samples short columns (10-15 cm) are recommended to reduce method development time. Such columns afford shorter retention time. In this work non polar column C<sub>18</sub> was used. It was used for the determination of Erlotinib HCl because of good separation.

## Selection of mobile phase

The selection of ideal mobile phase is done by altering the ratio of the buffers, composition of the mobile phase solvents in the different ratio and also by passing them through the column. Based on the literature survey and other experimental parameters finally the best suitable mobile phase (acetonitrile (50%): buffer (50%)) for the drug Erlotinib hydrochloride is selected for the method development.

## Mobile phase preparation

Acetonitrile and phosphate buffer were mixed in the ratio of 50: 50 v/v and sonicated for 15 min for degassing. The solution was filtered through 0.45 µm membrane filter.

## Preparation of buffer solutions

### pH 1.4 Buffer:

The buffer was prepared by mixing 6.57 g of potassium chloride with 119.0 ml of 0.1 M hydrochloric acid and diluted up to 1000 ml with milli Q water. Then pH was adjusted to 1.4 with hydrochloric acid. 250 ml of 0.1 M hydrochloric acid was prepared by mixing 2.25 ml of 37% hydrochloric acid with milli Q water.

### pH 2.4 Buffer:

It was prepared by mixing 6.7 ml of orthophosphoric acid with 50.0 ml of 4% v/v solution of 2 M sodium hydroxide and diluted to 1000 ml with demineralized water. pH was adjusted to 2.4 with sodium hydroxide. 100 ml of 2 M sodium hydroxide was prepared by dissolving 8.0 g of sodium hydroxide in demineralized water and standardized with oxalic acid.

### pH 4.5 Buffer:

Dissolve 5.4 gm of sodium acetate and 3.35 ml of glacial acetic acid and dilute with water to 100 ml.

## Mobile phase

Buffer preparation: Accurately weighed 1.9 g of potassium di-hydrogen orthophosphate transferred in 500 ml of milli Q water and 1 ml of triethylamine was added and adjusted the pH to 2.4 by dilute ortho phosphoric acid solution and made the volume upto 1000 ml with purified water.

### ***In vitro* drug-drug interaction study**

#### **Preparation of stock solutions for proton pump inhibitors**

Standard stock solution of pantaprazole, omeprazole and rabeprazole of 1000 µg/ml were prepared by dissolving 100 mg of each pantaprazole, omeprazole and rabeprazole in 20 ml of methanol in a 100 ml volumetric flask separately. The above solution was sonicated for 10 minute and the volume was made upto 100 ml with the methanol. The standard stock solution (10 ml) was diluted to 100 ml with the different buffers like 1.4, 2.4 and 4.5 to get the final concentration of 100 µg/ml for all the PPI. Series of dilution were made to get concentration range 50 µg/ml.

#### **Method I**

##### **Drug-drug interaction study using aqueous medium by RP-HPLC method**

High-performance liquid chromatography is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture. Retention time and absorbance peak of the species in solution may be changed due to the interaction with other species (Saeed A. et al., 2010). In the present study, analysis of Erlotinib hydrochloride with proton pump inhibitors were carried out, using HPLC (Shimadzu, Japan) at pH 1.4, 2.4 and 4.5 with a concentration of 50 µg/ml where combination of Erlotinib hydrochloride with proton pump inhibitors was 1:1 molar ratio (50 µg/ml). The studies were repeated twice.

#### **Procedure**

Stock solutions of Erlotinib hydrochloride and PPIs were prepared in methanol and the concentrations are 100 µg/ml. These stock solutions are diluted separately with different pH buffers (pH 1.4, 2.4 and 4.5) to get final concentration of 50 µg/ml. The stock solutions of Erlotinib hydrochloride and PPIs were mixed in the ratio 1:1 in 10 ml volumetric flask by taking 5 ml of each solution and sonicated for 15 minutes, separately. After sonication these solutions are filtered through membrane filter 0.45 µm and injected to HPLC for analysis of sample. Based on the difference in retention time of Erlotinib hydrochloride without PPI and with PPI, interactions of drug is judged and reported.

## Method II

### Extraction of plasma from rat blood

Blood samples (2 ml) were collected in evacuated glass tubes from healthy rats (6 animals: Institutional animal ethics committee under proposal no IAEC/PH,ANA/03/2016) by retroartery route method. The blood was centrifuged at 1500 rpm for 10 mins and the supernatant plasma was separated using micropipette. The separated plasma is deproteinated using methanol. The supernatant obtained was filtered through 0.45  $\mu\text{m}$  membrane filter. Collected plasma is mixed with drug stock solution prepared in buffer at pH 1.4, 2.4 and 4.5 in 1:1 ratio in eppendorf tubes. To the mixture (500  $\mu\text{l}$ ), 4.5 ml of methanol was added and centrifuged at 1500 rpm for 10 mins. After centrifugation the protein present in serum is precipitated and the supernatant layer is collected, filtered and placed in petridish. This solution is allowed to dry for 4 hours till methanol get evaporated. After drying, the remaining drug on petridish is redissolved in 1 ml of methanol. This solution is filtered using 0.45  $\mu\text{m}$  filter and injected for HPLC analysis. Based on the difference in retention time of Erlotinib hydrochloride without PPI and with PPI, drug-drug interactions are judged and reported.

## Results and Discussion

### *In vitro* drug-drug interaction study in aqueous medium

Analysis of Erlotinib hydrochloride with proton pump inhibitors was carried out, using HPLC. The pH at 1.4, 2.4 and 4.5 with a concentration of 50  $\mu\text{g/ml}$  was maintained, where combination of Erlotinib hydrochloride with proton pump inhibitors was 1:1 molar ratio (50  $\mu\text{g/ml}$ ). From the obtained results, *in vitro* drug-drug interaction studies at pH 1.4, pH 2.4 and pH 4.5 there is no change in the peak shift or gradual change in the retention time of Erlotinib hydrochloride with PPI and without PPI combinations. So there is no possible interactions seen during our experiment at *in vitro* conditions in aqueous medium.

Erlotinib hydrochloride alone at pH 1.4 in aqueous medium, retention time has shown at 3.88. And with PPIs (1:1), the retention time was found at 3.87 (pantaprazole), 3.86 (Omeprazole) and 3.88 (Pantaprazole). At pH 2.4 in aqueous medium, Erlotinib HCL has shown retention time at 3.85 and with PPIs (1:1), the retention time was found at 3.86 (Rabeprazole) and 3.85 (Omeprazole and pantaprazole). At pH 4.5 in

aqueous medium, retention time was 5.22 and with PPIs (1:1), the retention time was 5.42 (Rabeprazole), 5.14 (Omeprazole) and 5.20 (Pantaprazole, Table 1) respectively.

### ***In vitro* drug-drug interaction study in rat plasma**

From the obtained results, after performing *in vitro* drug-drug interaction studies at pH 1.4, 2.4 and 4.5, it has mixed with plasma. The observed interaction study is no shift or gradual change in the  $R_t$  of the Erlotinib hydrochloride with PPIs and without PPIs. Hence, there are no possible interactions seen during our experiment at *in vitro* conditions (Satheeshmanikandan R.S., 2012).

In drug-drug interaction study, Erlotinib hydrochloride at pH 1.4 in plasma, retention time has shown at 3.83 and Erlotinib hydrochloride with PPIs (1:1), the retention time was found at 3.87 (pantaprazole) and 3.85 (Omeprazole and rabeprazole). At pH 2.4 in plasma, retention time has shown at 3.85 and with PPIs (1:1), the retention time was found at 3.85 (Rabeprazole) and 3.86 (Omeprazole and pantaprazole). At pH 4.5 in aqueous medium, retention time has shown at 5.22 and with PPIs (1:1), the retention time was found at 5.14 (Rabeprazole), 5.41 (Omeprazole) and 5.16 for pantaprazole (Table 2).

It can be concluded that Erlotinib hydrochloride does not form any stable complex with PPI. Therefore, mentioned results may consider during monitoring and concurrent therapy of both drugs. The developed method has good approach for obtaining reliable results and was found to be suitable for the routine estimation of Erlotinib hydrochloride.

### **Acknowledgement**

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### **Figures:**

FIG1: Chromatogram of of Erlotinib hydrochloride at pH 1.4.

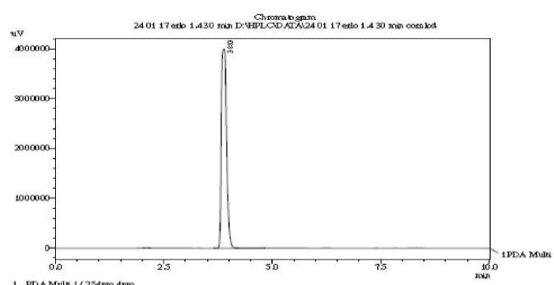


FIG 2: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Pantaprazole at pH 1.4.

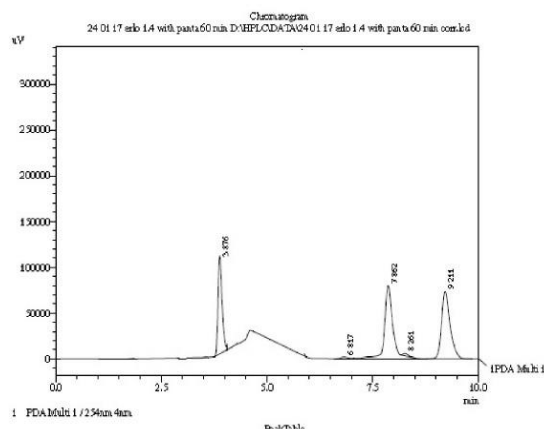


FIG 3: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Rabeprazole at pH 1.4.

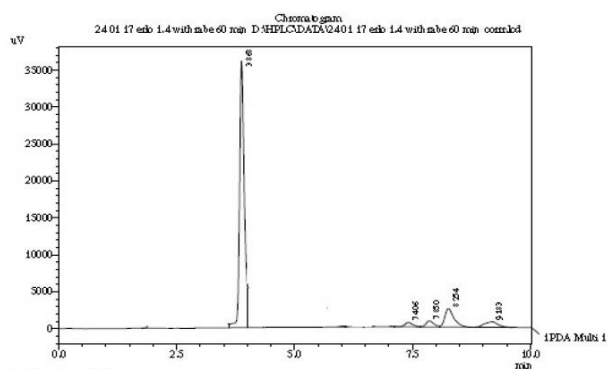


FIG 4: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Omeprazole at pH 1.4.

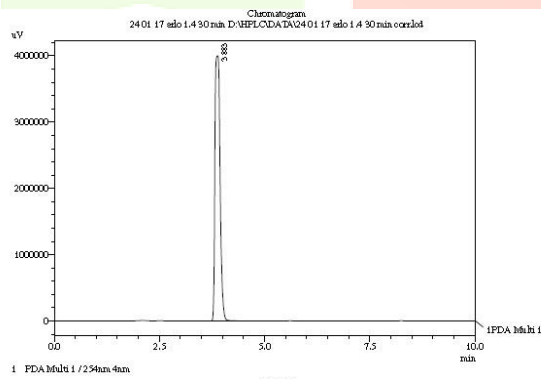


FIG 5: Chromatogram of Erlotinib hydrochloride at pH 2.4.

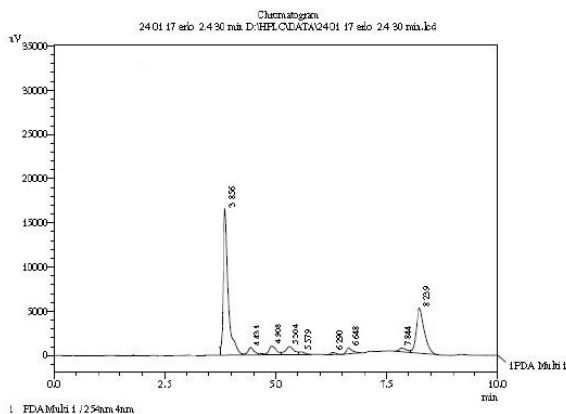


FIG 6: Chromatogram of *In vitro* drug-drug interaction Erlotinib hydrochloride at pH 2.4 with pantaprazole.

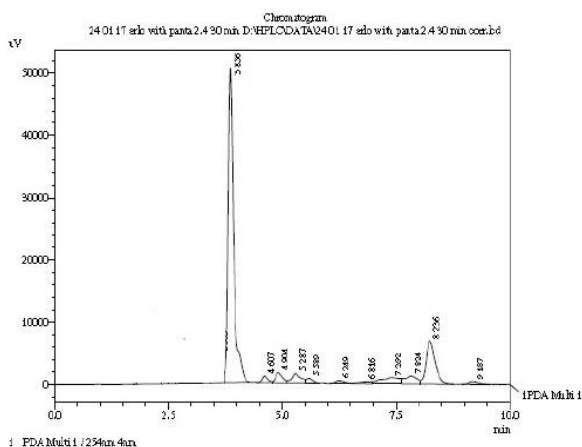


FIG 7: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Omeprazole at pH 2.4.

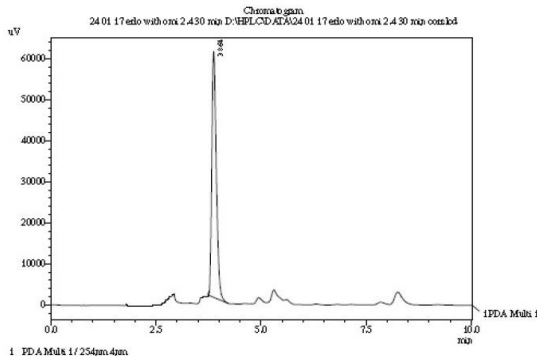


FIG 8: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Rabeprazole at pH 2.4

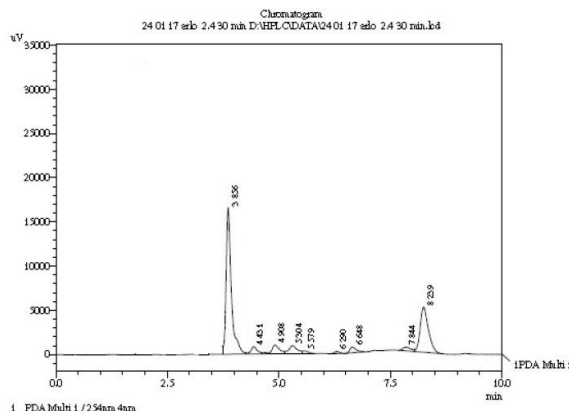


FIG 9: Chromatogram of Erlotinib hydrochloride at pH 4.5



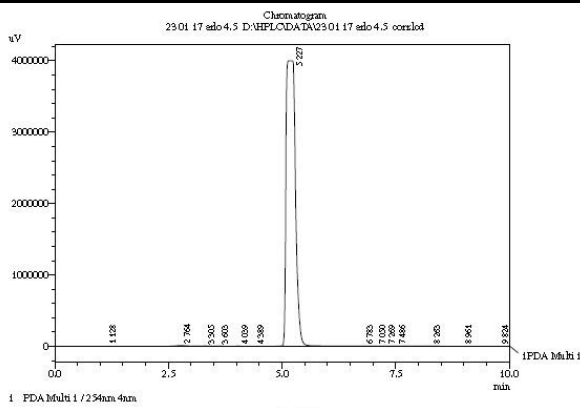


FIG 10: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with omeprazole at pH 4.5

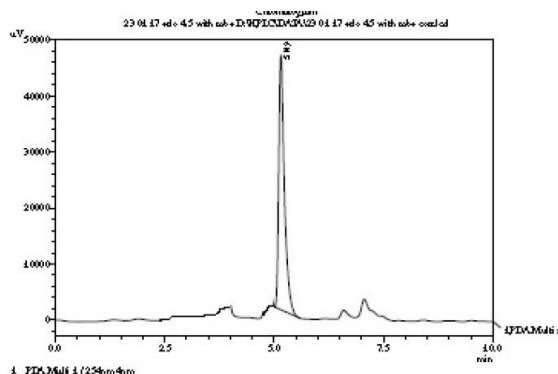


FIG 11: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Rabeprazole at pH 4.5

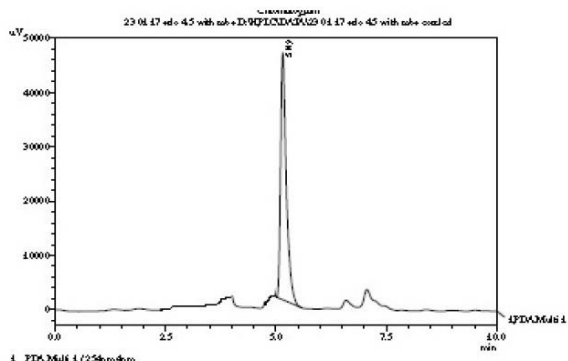


FIG 12: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Pantaprazole at pH 4.5.

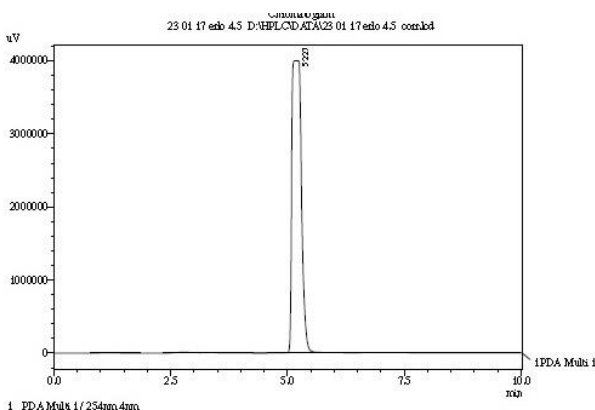


FIG 13: Chromatogram of Erlotinib hydrochloride in plasma at pH 1.4.

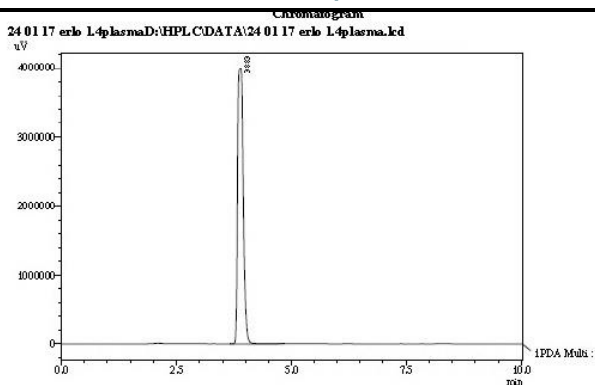


FIG 14: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Pantaprazole in plasma at pH 1.4.

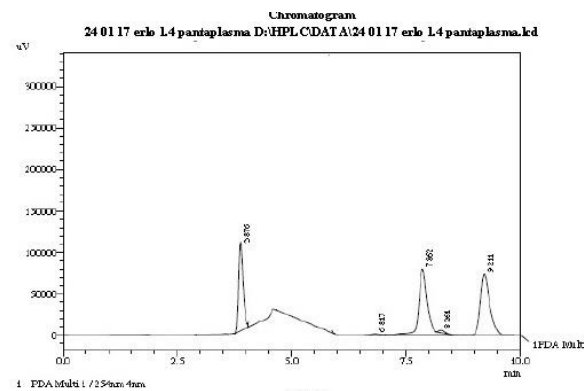


FIG 15: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Rabeprazole in plasma at pH 1.4.

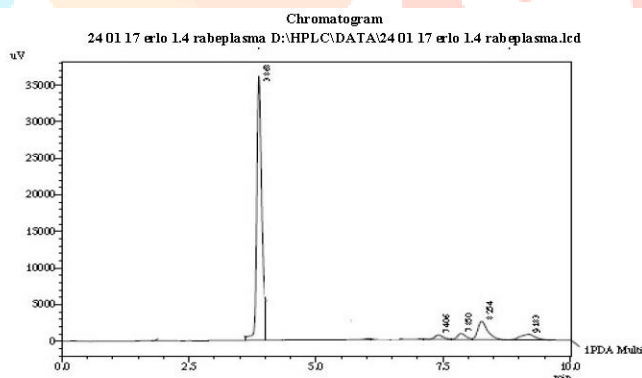


FIG 16: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Omeprazole in plasma at pH 1.4.

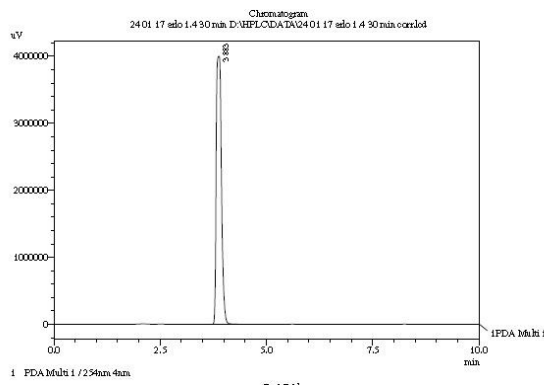


FIG 17: Chromatogram of Erlotinib hydrochloride in plasma at pH 2.4.

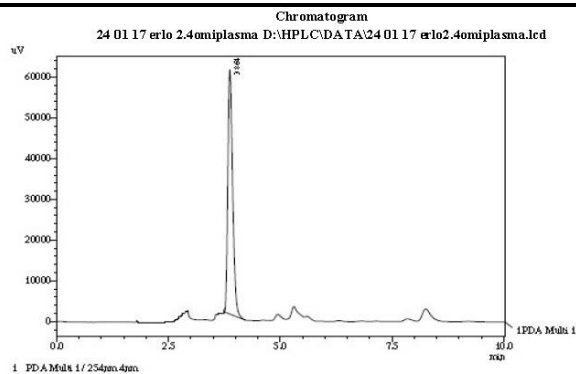


FIG 18: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with omeprazole in plasma at pH 2.4.

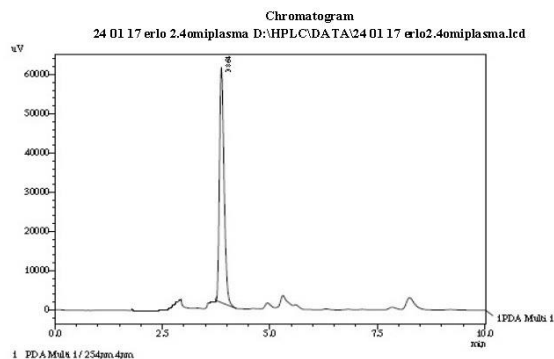


FIG 19: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Rabeprazole in plasma at pH 2.4.

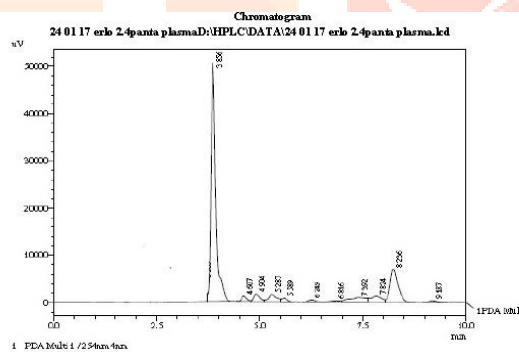


FIG 20: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with Pantaprazole in plasma at pH 2.4.

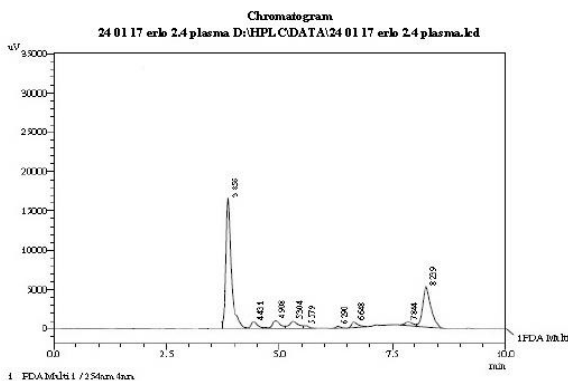


FIG 21: Chromatogram of Erlotinib hydrochloride in plasma at pH 4.5

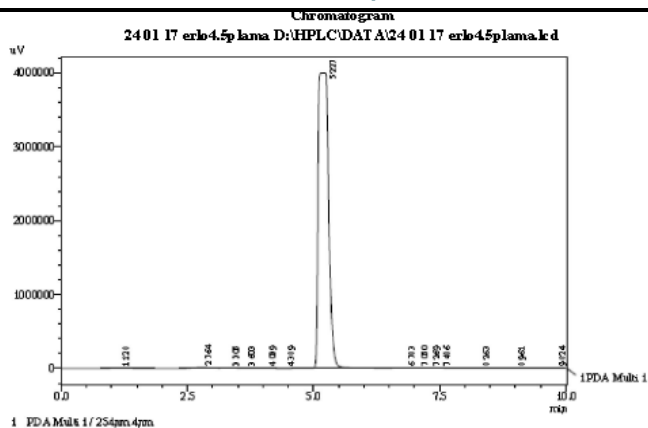


FIG 22: Chromatogram of *In vitro* drug-drug interaction of Erlotinib hydrochloride with omeprazole in plasma at pH 4.5.

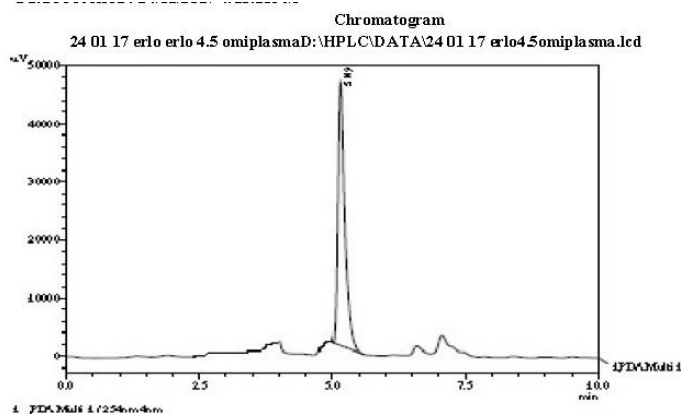


FIG 23: Chromatogram of *In vitro* drug-drug interaction of Erlotinib with Rabepazole in plasma at pH 4.5.

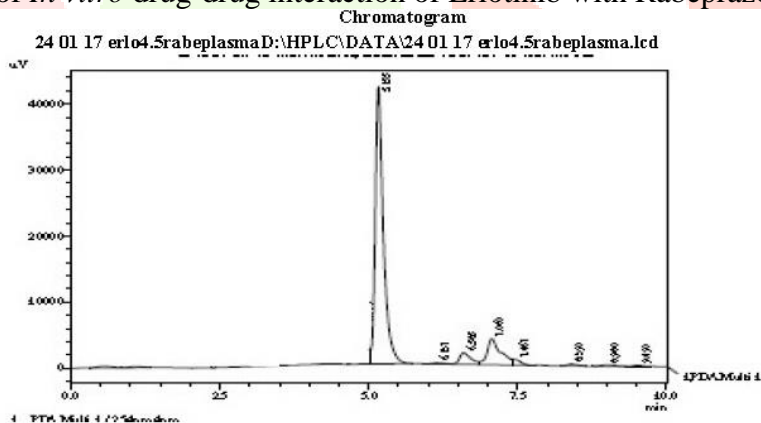
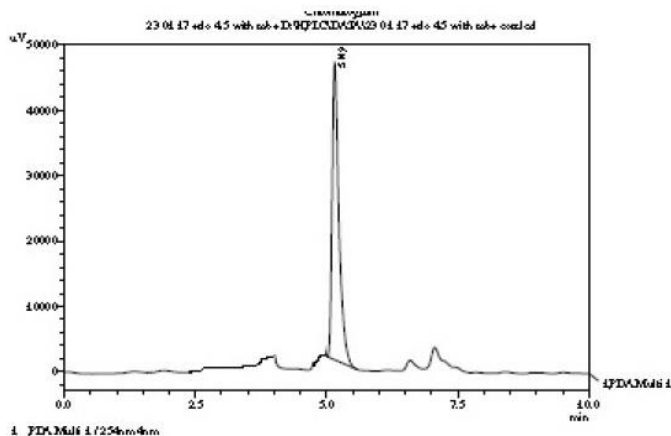


FIG 24: Chromatogram of *In vitro* drug-drug interaction of Erlotinib with Pantaprazole in plasma at pH 4.5



## Tables

**Table 1: *In vitro* drug-drug interaction of Erlotinib hydrochloride with proton pump inhibitors at pH 1.4, 2.4 and 4.5 in aqueous medium.**

Sl. No	Time in mins	Rt for Erlotinib hydrochloride	Rt for Erlotinib hydrochloride with Pantaprazole	Rt for Erlotinib hydrochloride with Omeprazole	Rt for Erlotinib hydrochloride with Rabeprazole
<b>At PH 1.4</b>					
1	0	3.88	3.87	3.86	3.86
2	30	3.82	3.87	3.85	3.87
3	60	3.84	3.85	3.87	3.85
<b>At pH 2.4</b>					
1	0	3.85	3.87	3.86	3.88
2	30	3.84	3.86	3.85	3.87
3	60	3.85	3.85	3.84	3.89
<b>At pH 4.5</b>					
1	0	5.22	5.34	5.14	5.41
2	30	5.23	5.34	5.15	5.44
3	60	5.20	5.38	5.14	5.42

**Table 2: *In vitro* drug-drug interaction of Erlotinib hydrochloride with proton pump inhibitors in plasma at pH 1.4, 2.4 and 4.5.**

Sl. No	Time in mins	Rt for Erlotinib hydrochloride	Rt for Erlotinib hydrochloride with Pantaprazole	Rt for Erlotinib hydrochloride with Omeprazole	Rt for Erlotinib hydrochloride with Rabeprazole
<b>pH 1.4</b>					
1	0	3.83	3.87	3.85	3.85
2	30	3.86	3.82	3.86	3.86

3	60	3.83	3.84	3.85	3.84
<b>pH 2.4</b>					
1	0	3.85	3.86	3.86	3.85
2	30	3.84	3.87	3.86	3.86
3	60	3.86	3.85	3.84	3.82
<b>pH 4.5</b>					
1	0	5.22	5.16	5.41	5.14
2	30	5.21	5.14	5.42	5.14
3	60	5.23	5.18	5.42	5.14

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