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“Stability Indicating RP–HPLC Method for The Estimation of Etravirine in Pure and Tablet Dosage Form.”

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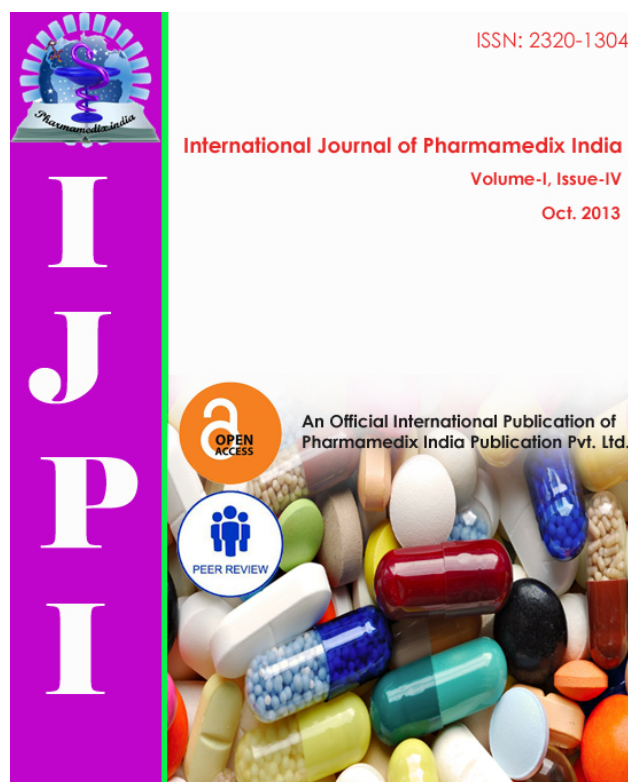
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Abstract:

A simple, fast and precise stability indicating RP – HPLC method was developed for the separation and quantification of Etravirine in pharmaceutical dosage form. The quantification was carried out using Universal C18 4.6X150mm, 5µm enhanced polar selectivity column and mobile phase comprised of 0.02M potassium dihydrogen phosphate & 0.003M di potassium hydrogen phosphate buffer pH adjusted to 3.5 with 0.1% orthophosphoric acid and acetonitrile in proportion of ratio 45:55 and degassed under ultrasonication. The flow rate was 1mL/min and the effluent was monitored at 285nm. The retention time of Etravirine was 4.547min. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantitation in accordance with ICH guidelines. Linearity of Etravirine was in the range of 10 – 60 µg/mL. The average percentage recovery of Etravirine was 99.02% from the tablet formulation. The stability – indicating capability was established by forced degradation experiments. The proposed method is suitable for determination of Etravirine in pharmaceutical dosage form.

Keyword: Chromatography, Etravirine, Forced degradation, Method development, Validation.

Introduction

Etravirine is chemically 4-[6-Amino-5-bromo-2- [(4-cyanophenyl)amino] pyrimidin-4-yl]oxy- 3,5 dimethylbenzonitrile. Etravirine is a Non-nucleoside reverse transcriptase inhibitor used in the treatment of HIV. It may be used alone or in combination with other Anti – HIV drugs.

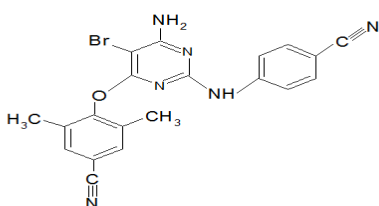


Figure 1 Structure of Etravirine

The objective of this work is to develop an analytical HPLC procedure, which will serve as stability indicating method for Etravirine. A few chromatographic methods have appeared in the literature for the quantification of Etravirine by HPLC, LCMS and UP – LC ^[1-7]. A through literature search revealed none of the reported analytical procedures describe a stability indicating HPLC method for the determination of Etravirine. To establish the stability indicating nature of the method, forced degradation of drug substance & drug product was performed under stress conditions (thermal,

acid, base and oxidative), and stress samples were analysed by proposed method ^[8]. The developed LC method is validated with respect to specificity, LOD, LOQ, linearity, precision, accuracy, and robustness as per ICH recommended conditions ^[9,10]. From the best of our knowledge via literature search, this is the first known stability indicating RP – HPLC method.

Methods:

Materials, reagents and chemicals

Etravirine is obtained as gift sample from Hetero Pharma Ltd, Hyderabad. HPLC grade acetonitrile and analytical grade potassium dihydrogen phosphate, di potassium hydrogen phosphate, ortho phosphoric acid obtained from Quagins Fine Chemicals Ltd, Mumbai. Hydrochloric acid, sodium hydroxide, hydrogen peroxide of analytical grade was obtained from Merc Chemicals Ltd, Mumbai.

Chromatographic conditions

Shimadzu HPLC with PDA detector was used. The output of signal was monitored and integrated using LC – solutions 2000 software. Universal C18 (4.6 × 150mm, 5µm) column was selected as stationary phase. Mobile phase comprised of 0.02M potassium di hydrogen phosphate, 0.003M di potassium hydrogen phosphate pH adjusted to 3.5 with 0.1% ortho phosphoric acid and acetonitrile in the ratio 45:55. Mobile phase was mixed, filtered through 0.45µm membrane filter and degassed under ultrasonication. The mobile

phase was used as diluents. Injection volume was 10µl and flow rate was 1ml/min. The column was maintained at ambient temperature and the eluent was monitored at 285nm.

Preparation of standard solution

A stock solution (100µg/ml) of Etravirine was prepared by dissolving 10 mg of drug taken in 100 ml volumetric flask, dissolved in 50ml of mobile phase, sonicated for 15 min and the volume was made up to 100 ml with mobile phase.

Preparation of working standards

The working standard solutions of Etravirine were prepared by accurately transferring the (1, 2, 3, 4, 5, 6 ml) aliquots of the standard stock solution in a series of 10 ml volumetric flasks. The volume was made up to mark with mobile phase to obtain concentration of 10 – 60 µg/ml.

Preparation of sample solution

10 tablets were accurately weighed and powdered. Weight equivalent to 25mg was weighed and taken in 50ml volumetric flask. Mobile phase (30 ml) was added and sonicated for 15min. The solution was filtered through Whatman filter paper No.1 and the volume was adjusted up to the mark with mobile phase to 1ml of resulting solution was taken in 10ml volumetric flask and volume made up to mark with mobile phase to obtain 50 µg/ml of sample solution.

Results and discussion:

System Suitability:

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0 , tailing factor ≤ 2.0 and theoretical plates >2000 . In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was $< 2.0\%$. The system suitability test was performed using five replicate injections of standards before analysis of samples. The results are summarized in table – 1.

Linearity:

Standard curves were constructed daily, for three consecutive days (Twice a day), using six standard concentrations in a range of 10, 20, 30, 40, 50 and 60 $\mu\text{g/ml}$ for Etravirine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The results are summarized in table – 2.

Precision:

System precision:

To study precision, six replicate standard solutions of Etravirine ($50\mu\text{g/ml}$) were prepared and analyzed using the proposed method. The percent relative standard

deviation (% RSD) for peak responses was calculated.

Method precision

The intraday and inter-day precision of the proposed method was determined by analyzing the corresponding responses 6 times for concentration of sample solutions of $50\mu\text{g/ml}$. Percentage assay was calculated and the result was reported in terms of relative standard deviation (% RSD).

Intermediate precision

The intermediate precision of the proposed method was determined by performing the method by two analysts for concentration of sample solutions $50\mu\text{g/ml}$. The percent relative standard deviation (% RSD) for peak responses was calculated.

Limit of Detection & Limit of Quantification

LOD and LOQ of drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ is the standard deviation of response

S is slope of the calibration curve

Accuracy (Recovery study)

The accuracy of the method was determined by calculating recovery of Etravirine by the standard addition method. Known amounts of standard solutions of Etravirine ($5\mu\text{g/ml}$) were

added to pre analysed sample solutions (40, 50, 60 5µg/ml) of Etravirine. The closeness of obtained value to the true value indicates that the proposed method is accurate.

$$\% \text{ Recovery} = [(C_t - C_{pa}) / C_s] \times 100$$

Where C_t = Total concentration of analyte

C_{pa} = Concentration of pre-analysed sample

C_s = Concentration of standard added to pre-analysed sample.

The results are summarized in table – 3.

Robustness:

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength. After each change, sample solution was injected and checked for % RSD of Area. The results are summarized in table – 4.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine Etravirine in their tablet dosage form. The results are summarized in table – 5.

Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24hr at room temperature. The results show that for both solutions, the retention time and peak area of Etravirine

remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of proposed method. The results are summarized in table – 6.

Acid degradation sample

10 tablets were accurately weighed and powdered. Weight equivalent to 25mg of Etravirine was taken in a 50mL clean dry volumetric flask add about 30mL of mobile phase and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 5mL of 0.1N acid (Hydrochloric acid), refluxed for 60minutes at 80°C, then cooled to room temperature, neutralize with 0.1N base (Sodium hydroxide) and dilute to volume with mobile phase. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with mobile phase.

Base degradation sample

10 tablets were accurately weighed and powdered. Weight equivalent to 25mg of Etravirine was taken in a 50mL clean dry volumetric flask add about 30mL of mobile phase and sonicate to dissolve it for about 30minutes with intermittent shaking at

controlled temperature. Then add 5mL 0.1N base (Sodium hydroxide), refluxed for 60minutes at 80°C, then cooled to room temperature, neutralize with 0.1N acid (Hydrochloric acid) and dilute to volume with mobile phase. Filter about 5mL of the above sample solution through 0.45μ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with mobile phase.

Thermal degradation sample

10 tablets were accurately weighed and powdered. This powder is exposed to 105°C for 5 days. Weight equivalent to 25mg of Etravirine was taken in a 50mL clean dry volumetric flask add about 30mL of mobile phase and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. And dilute to volume with mobile phase. Filter about 5mL of the above sample solution through 0.45μ

membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with mobile phase.

Peroxide degradation sample

10 tablets were accurately weighed and powdered. Weight equivalent to 25mg of Etravirine was taken in a 50mL clean dry volumetric flask add about 30mL of mobile phase and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 2ml of 30% peroxide and refluxed for 60minutes at 80°C, then cooled to room temperature. The volume was made up to mark with mobile phase. . Filter about 5mL of the above sample solution through 0.45μ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with mobile phase.

Table 1: System suitability parameters for Etravirine

S.No	Parameter	Observation
1	Peak asymmetric factor	1.147
2	No. of theoretical plates	6470
3	Retention time	4.547min

Table 2: Linearity study for Etravirine

S.No	Concentration (μg/ml)	Area (mV.sec)n= 6
1	10	463.865
2	20	861.430

3	30	1220.295
4	40	1610.589
5	50	2010.052
6	60	2395.830

Table 3: Recovery data for Etravirine

S.NO	Pre Analysed Sample Concentration (µg/ml)	Spiked amount (µg/ml)	% Recovery	Mean % Recovery	% RSD
1	40	5	98.56	98.74	0.244
2	40	5	99.01		
3	40	5	98.64		
4	50	5	98.69	99.030	0.299
5	50	5	99.17		
6	50	5	99.23		
7	60	5	99.30	99.28	0.017
8	60	5	99.27		
9	60	5	99.27		

Table 4: Robustness study of Etravirine

Parameter	Area (mV.sec) n =5	% RSD
Flow rate (ml/min)		
0.9ml/min	2113.937	0.0349
1.1ml/min	1852.255	0.0041
Wavelength (nm)		
283nm	2053.729	0.0065
287nm	2092.549	0.0055

Table 5: Assay of Etravirine

S.NO	Dosage	Sample concentration (µg/ml)	Sample estimated (µg/ml)	%Assay
1	50mg	50	49.848	99.696

Table 6: Stability studies of Etravirine

Stress Condition	Duration of stress	Area (mV.sec)	% Degradation	% of Active ingredient
Standard	-----	2010.052	-----	-----
Acid (0.1N HCl)	60 min	2002.421	0.380	99.620
Base (0.1N NaOH)	60 min	1983.074	1.343	98.657
Thermal (105 °C)	5 days	2001.542	0.424	99.576
Peroxide (30%)	60 min	2004.573	0.273	99.727

Table: 7 Summary of validated parameters for proposed method

Parameter	Result
Linearity range	10 – 60 µg/ml
Regression equation	$y = 38.56x + 77.41$
Slope	38.56
Intercept	77.41
Correlation coefficient	0.999
System precision (% RSD, n=5)	0.335
Method precision (% RSD, n=5)	0.211
Intermediate precision (% RSD, n=5)	0.125
LOD (µg/ml)	0.0035
LOQ (µg/ml)	0.0117
% Recovery (Accuracy, n =3)	0.299
% Assay (% Assay, n=5)	99.696

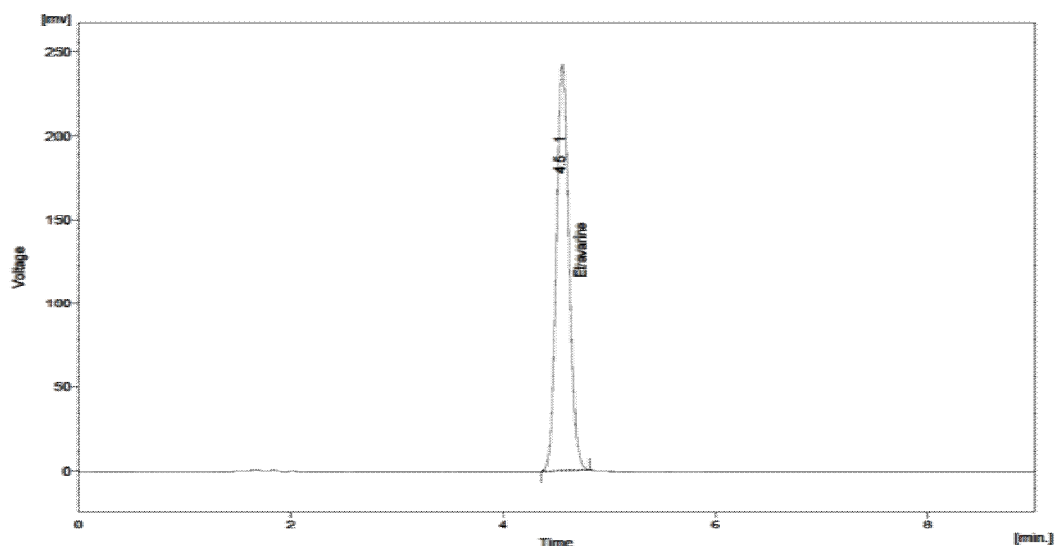


Figure 2: Standard chromatogram of Etravirine

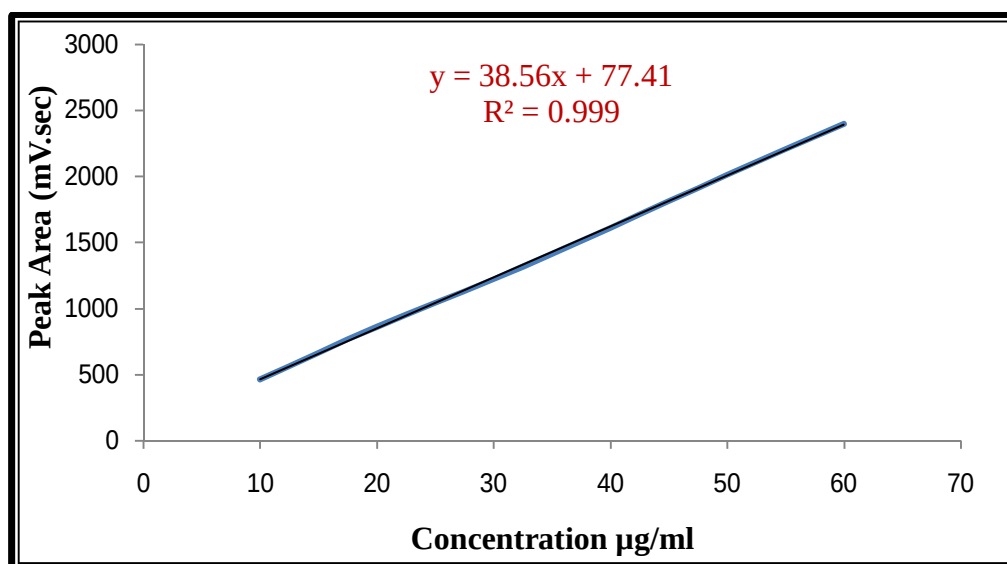


Figure 3: Calibration curve of Etravirine

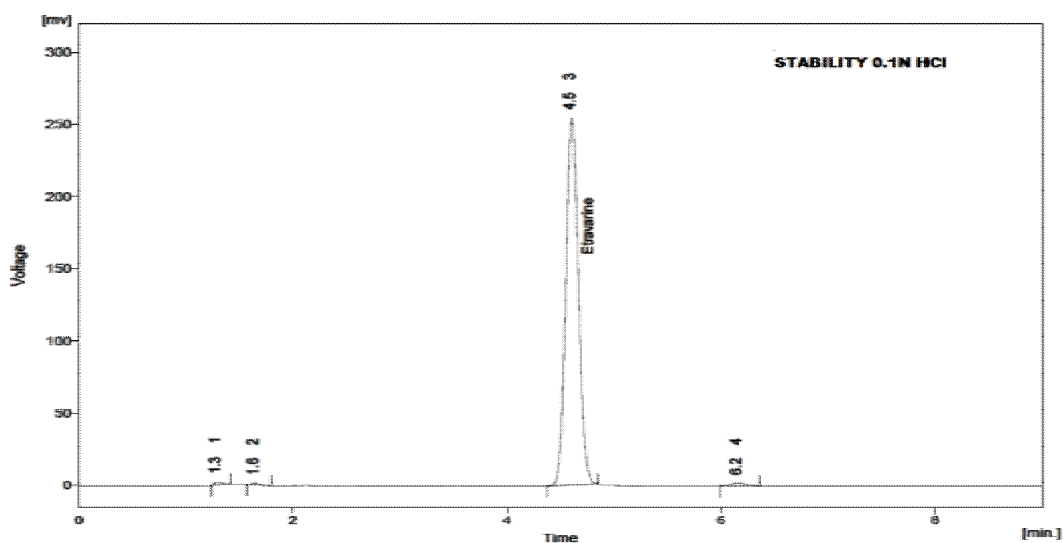


Figure 4: Stability studies using 0.1N HCl

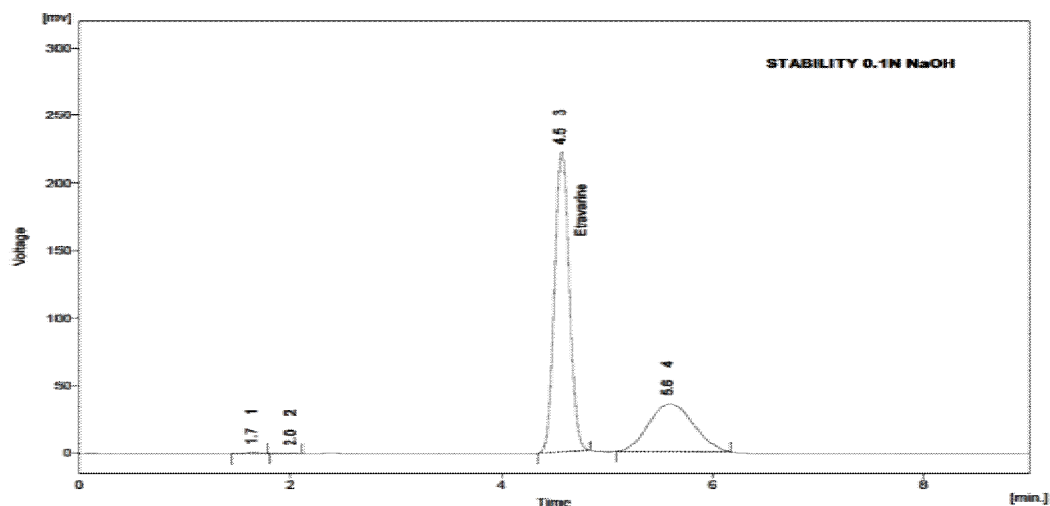


Figure 5: Stability studies using 0.1N NaOH

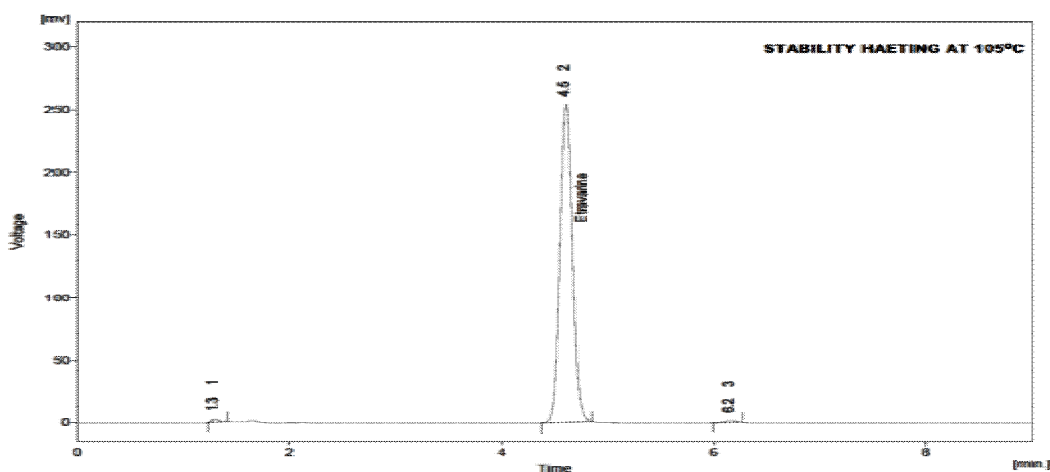


Figure 6: Stability studies at 105°C

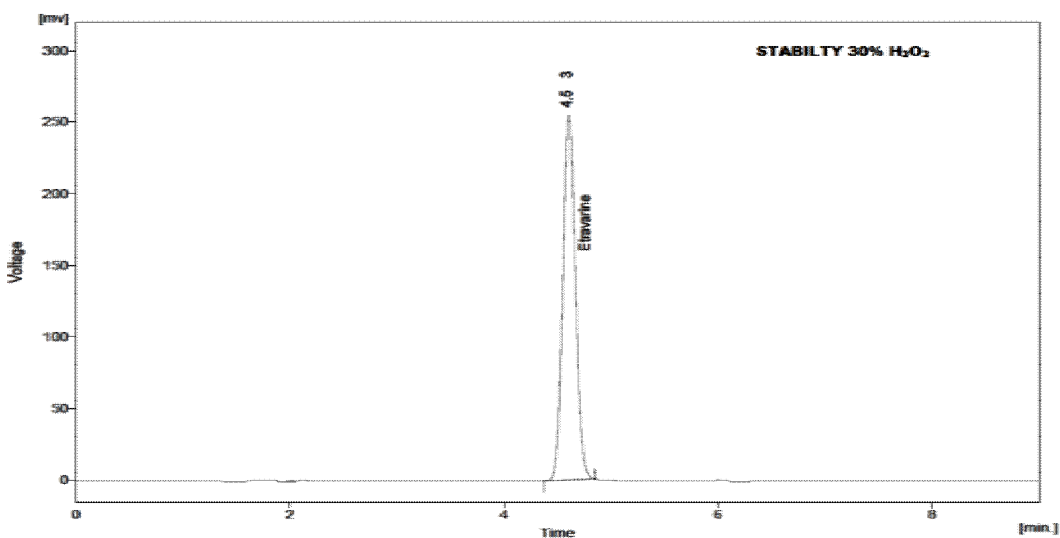


Figure 7: Stability studies using 30% H₂O₂

Conclusion

Thus the proposed Stability indicating RP-HPLC method for the determination of Etravirine in tablet dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of pharmaceutical formulation of Etravirine in tablet formulation.

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