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Full Length Research Paper

Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form

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A rapid and accurate isocratic HPLC method was developed and validated for the assay of Efavirenz (EFA) in bulk and pharmaceutical dosage forms. The chromatographic conditions comprise of a Novapak phenyl column. A mixture of phosphate buffer and Acetonitrile was used as mobile phase. Quantitation was achieved by UV detection at 247 nm. A linear response (r^2 0.999) was observed in the range of 0.05 - 0.15 mg/mL. The method was validated for accuracy and precision. The proposed method can be used for quality control assay of EFA in bulk and in finished dosage form and for the stability studies as the method separates EFA from its degradation products and excipients.

Key words: Efavirenz, stability indicating HPLC, dosage form, UV detection.

INTRODUCTION

Chemically, efavirenz is (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-,1-benzoxazin-2-one. EFA is a non-nucleoside reverse transcriptaseinhibitor (NNRTI) and is used as a part of highly active antiretroviral therapy for the treatment of a human immunodeficiency virus (Figure 1). The drug is used in combination with other anti retroviral agents for the treatment of HIV-1 infection in children and adults. The usual dose of EFA is 600 mg per day (usually given at bed time).

Several methods have been reported for determination of efavirenz. Careri et al. (1993) achieved separation of alkynes by reversed phase HPLC using ruthenium complexes. Gita et al. (2008) and Agnes et al. (2008) reported separation of efavirenz in human plasma by using reversed phase HPLC technique using C18 column. So far in our knowledge only one stability indicating method has been reported using cyano column for the determination of efavirenz (Montgomery ER et al., 2001). The disadvantage of the method is that its run time is about 15 min and gradient separation. The Indian pharmacopoeia (Indian Pharmacopoeia, 2007) lso published isocratic HPLC method for the assay of EFA.

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The Run time is about 15 min. The present work describes a stability indicating LC method using phenyl column. The method is rapid, accurate and precise. The run time is of 6.6 min and the drug is well separated from all of its degradants. Therefore the method can be employed as stability -indicating one.

EXPERIMENTAL

Chemicals

A sample of EFA, assigned purity 99.3% of pharmaceutical grade was received from Arobindo Pharmaceuticals, Hyderabad, India. EFA film coated tablet of strength 600 mg Sustiva (Bristo-Myers Squibb-Germany) and Efavir (Cipla Ltd-India) were procured from the market. Potassium dihydrogen orthophosphate, dibasic sodium phosphate and sodium hydroxide of analytical grade were purchased from Qualigens (Mumbai India). HPLC grade acetonitrile were purchased from Merck (Mumbai, India) High purity water was prepared by Millipore milli Q plus purification system. (Millipore-France)

Equipment

The M/S Shimadzu Japan HPLC system with a photodiode array detector system (SPD –M20A) was used for the method development and forced degradation studies. The LC system used for method validation was Shimadzu HPLC LC- 2010CHT with

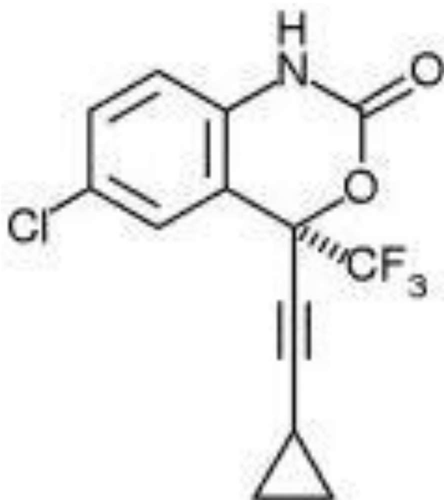

Figure 1. Chemical structure of Efavirenz.

quaternary gradient pumps. The out put signal was monitored and processed using LC-solution (Shimadzu-Japan) on Pentium computer (HCL-Mumbai).

Chromatographic conditions: The chromatographic column used was Novapak phenyl column, 150 x 3.9 mm dia with 4 particle size.(Waters-Ireland). The mobile phase comprised of mixture of buffer solution pH 6.0 and acetonitrile in proportion (55:45 v/v). The mixture of buffer solution was prepared by mixing 1:1 ratio of monobasic potassium phosphate buffer and dibasic sodium phosphate buffer.

The pH of the mixture was adjusted to 6.0 with orthophosphoric acid. The monobasic potassium phosphate buffer was prepared by dissolving 6.8 g of monobasic potassium phosphate and 1.7 g of sodium hydroxide in a liter of milli Q water and sodium dibasic phosphate buffer was prepared by dissolving 4 g of sodium dibasic phosphate in a liter of milli Q water. Acetonitrile was used as diluent.

The mobile phase was filtered through 0.45-micron membrane filter, degassed in ultrasonic bath and pumped from the respective solvent reservoir to the column at a flow rate of 1.0 mL min^{-1}. The column temperature was maintained at 30°C and the detection wavelength was 247 nm. The injection volume was 20 µL. The column was equilibrated for 60 min prior to the injection of the drug solution.

Preparation of standard solution

The standard stock solution of EFA 1.0 mg/mL was prepared by dissolving working standard in acetonitrile. The standard solution of 0. 1 mg/mL was prepared by dilution using stock standard solution in mobile phase.

Preparation of sample solution

To determine the content of EFA, twenty tablets were weighed and transferred into a clean and dry mortar. Then crushed and mixed well to prepare homogeneous mixture. A sample equivalent to 50 mg of EFA was taken in 50 mL volumetric flask with the aid of 25 mL acetonitrile and soniccated for 10 min diluted to 50 mL. Further 5 mL of this solution diluted to 50 mL with mobile phase (0.1 mg/mL). All the experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Method development

The HPLC procedure was optimized with a view to develop a stability- indicating assay method. Various columns like octadecyl silane column, Octadecasilyl column, amide column and ion exchange column have been tried with different buffer in combination with acetonitrile and methanol.

However, good resolution was obtained by using Novapak phenyl column with monobasic potassium phosphate buffer and dibasic sodium phosphate buffer at pH 6.0. Upon mixing both the buffer in 1:1 ratio and adding organic modifier acetonitrile obtained well defined peak shape of EFA. The mobile phase consisting of mixture of buffer and acetonitrile in proportion (55:45 v/v) was used with a flow rate of 1.0 mL min^{-1} on phenyl column, the retention time of EFA was about 6.5 min. EFA and its degradants were well separated.

Degradation studies

In an attempt to develop a stability indicating assay method, the samples of tablet powder were subjected separately for different conditions. Acidic degradation using 5 mL of 1N HCl, basic degradation using 5 mL 1N NaOH solution, Oxidative degradation using 5 mL of 3% hydrogen peroxide solution. These conditions all exposed at 80°C for 6 h. The forced degradation in acidic, basic and oxidation media performed in the dark in order to avoid the possible effect of light.

Photodegradation study was performed for the same samples by exposing them to artificial solar radiation of irradiation of 1,200 K lux of visible light and 200 W h m^{-2} of UV light by using photo stability chamber. Heat degradation was carried out by heating at 105°C for 12 h. All the degraded drug solutions after appropriate dilutions (0.1 mg/mL) with mobile phase (neutralization for the acidic and basic degradation) were injected in the chromatographic system. Typical chromatograms of all degraded tablet samples are shown in Figures 2 - 7. The degraded samples were compared to a tablet sample without degradation.

The spectral homogeneity (Peak purity) 200 - 400 nm was determined in the forced degraded samples. The threshold was set at 0.990. The peak purity, peak threshold and percent degradation for EFA in tablet were demonstrated that the proposed LC method was able to separate the drug from degradants enerated duringforced degradation studies (Table 1).

Linearity: The linearity of the response of EFA was verified at five concentration level ranging from 0.05 - 0.15 mg/mL (0.05, 0.08, 0.1, 0.12 and 0.15 mg/mL) . The calibration curve was constructed by plotting mean area response A against concentration C. The regression

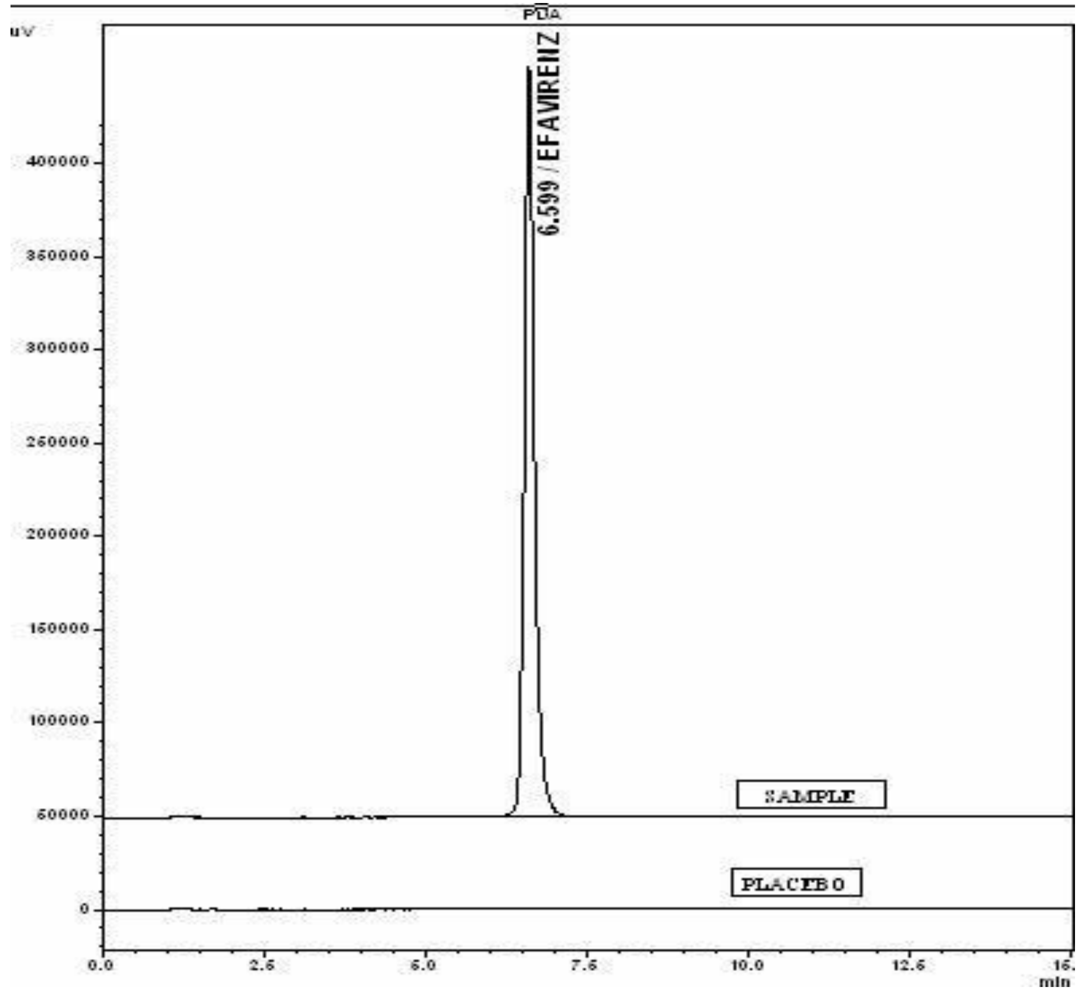

Figure 2. Chromatograms of untreated Efavirenz in tablet sample.

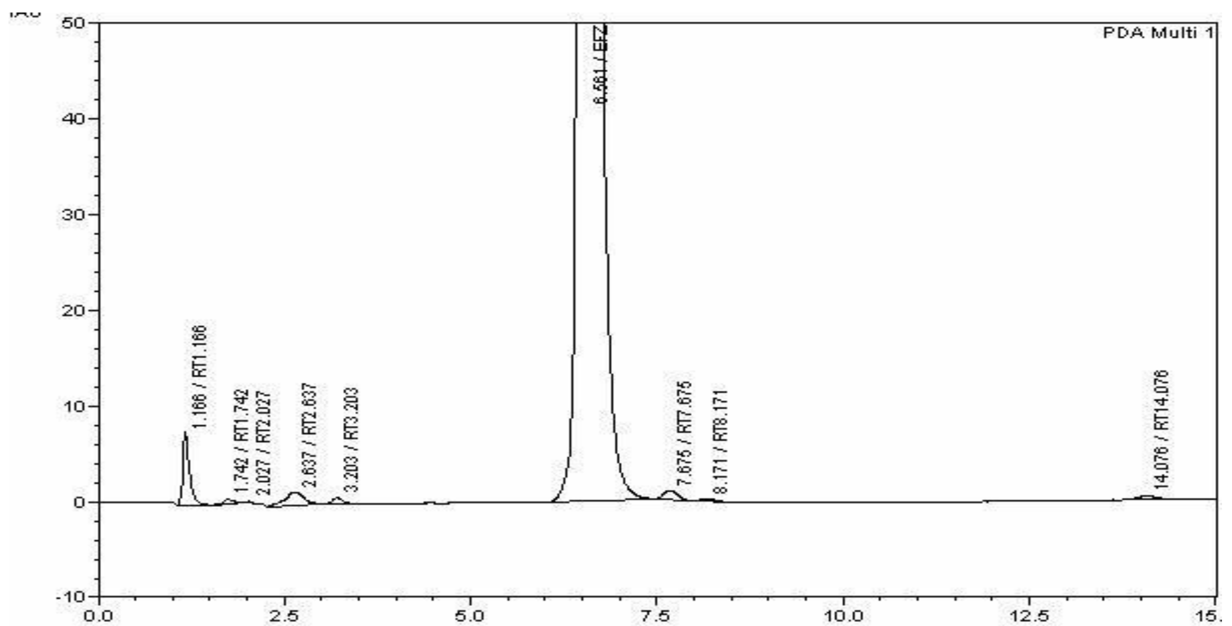

Figure 3. Chromatograms of acid hydrolysis–degraded EFA in tablet sample.

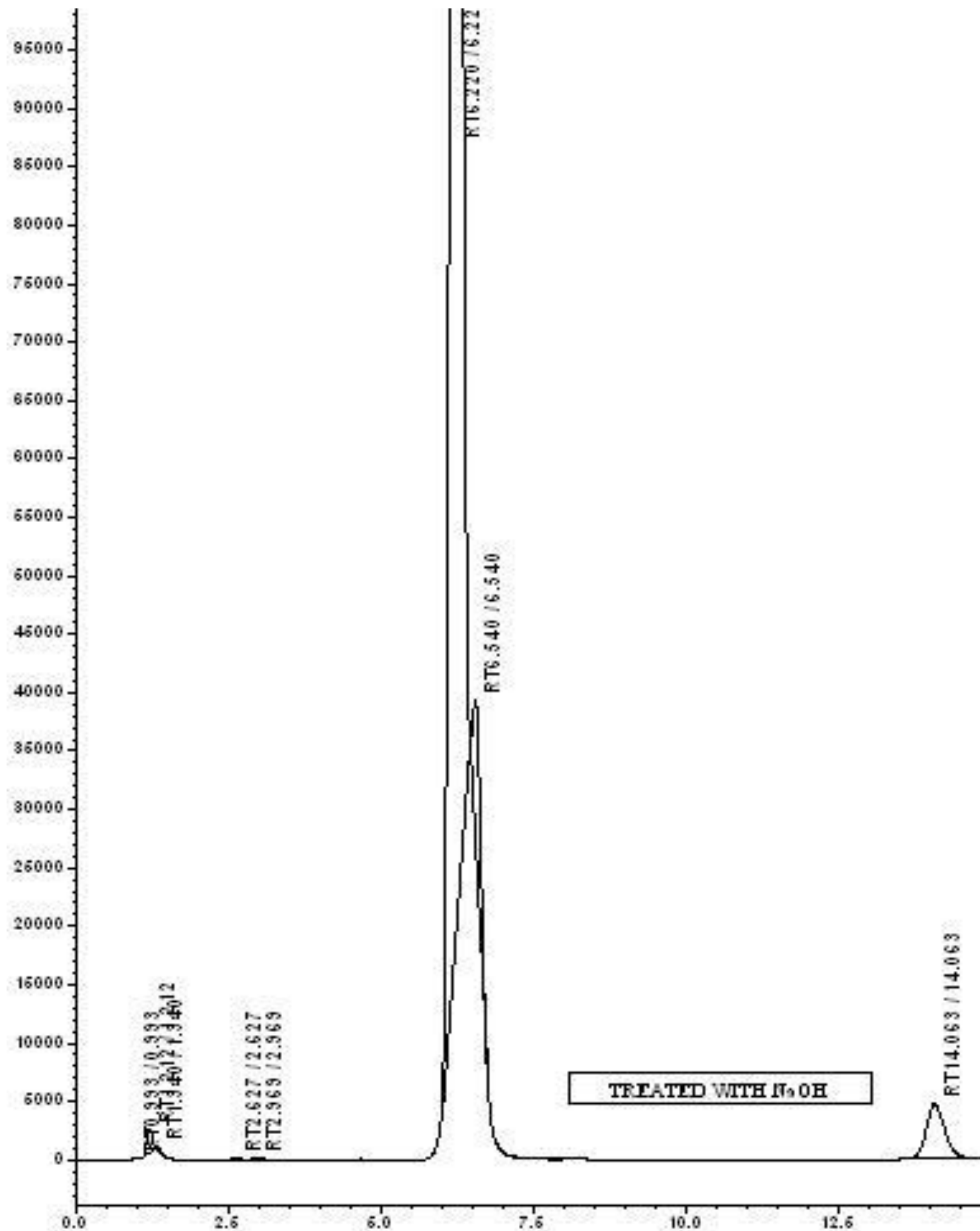

Figure 4. Chromatograms of base hydrolysis–degraded EFA in tablet sample.

equation obtained for the EFZ was 56336975C + 316007 (r^2 = 0.9999, n = 5).

The result show that an excellent correlation existed between peak area and concentration of EFA within the concentration range tested.

Precision

Method repeatability (intra-day precision) was evaluated by assaying five EFA tablet samples of same batch, which were prepared as described in the sample preparation. The mean % assay and percentage R.S.D. for assay values were found to be 99.0% and 0.7%, respectively, which is well within the acceptance criteria that is, assay value should be between 97.0 and 103.0% and R.S.D. should be not more than 2.0%. The intermediate precision (inter-day precision) was performed by assaying five EFA tablet samples prepared by different analyst, different HPLC system and different HPLC column in different days as described in the sample preparation. The mean % assay and percentage R.S.D.

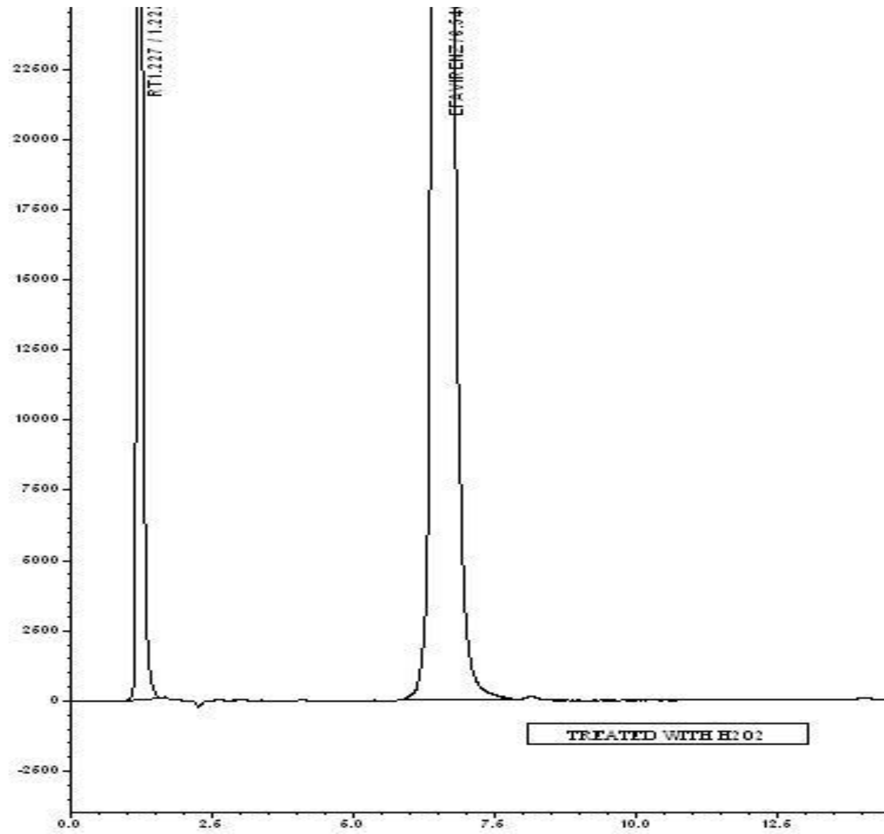

Figure 5. Chromatograms of oxidative–degraded EFA in tablet sample.

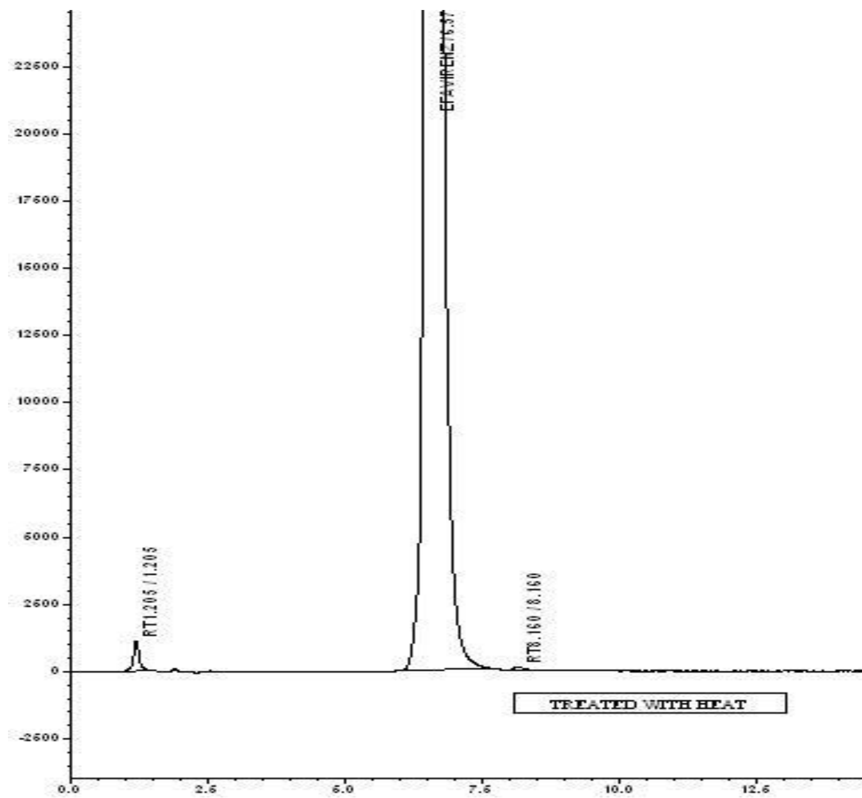

Figure 6. Chromatograms of thermal –degraded EFA in tablet sample.

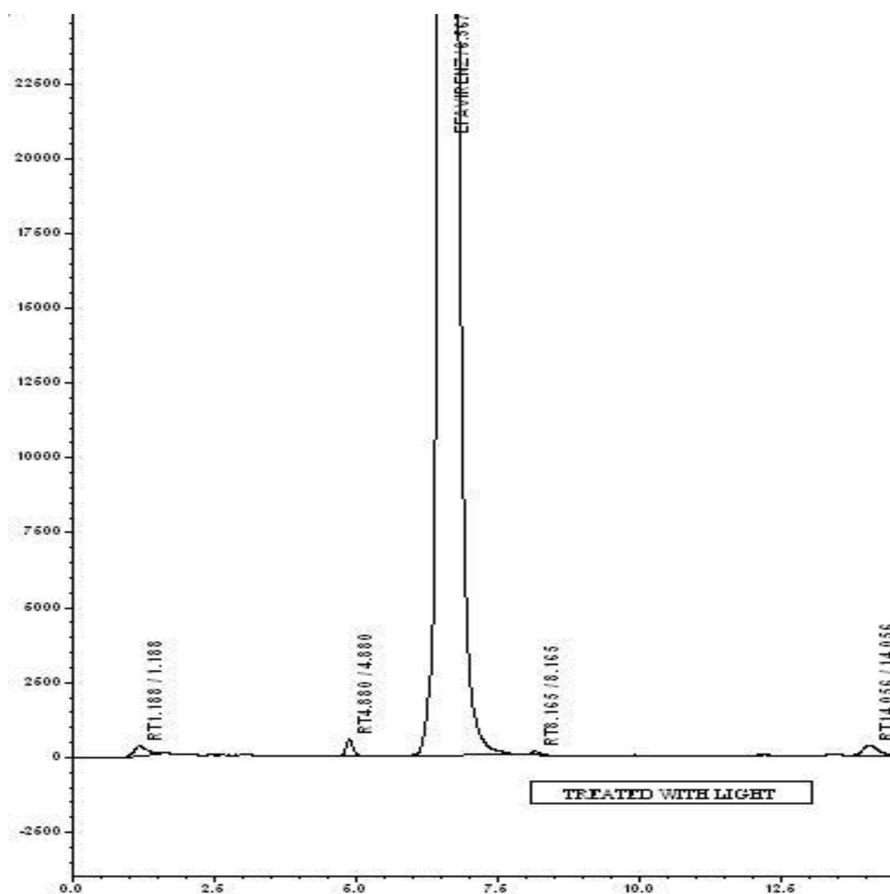

Figure 7. Chromatograms of photo degraded EFA in tablet sample

Table 1. Forced degradation Results of EFA in tablet sample.

Stress condition	Drug product			
	% degradation	Impurity	Peak purity index	Single point threshold
Sample without degradation	Nil	ND	1.000	0.9999
Refluxed with 1 N HCl solution for about 6 h at 80°C	1.75%	ND	1.000	0.9999
Refluxed with 1 N NaOH solution for about 6 h at 80°C	6.44%	ND	1.000	0.9999
Refluxed with 3% H_2O_2 solution for about 6 h at 80°C	6.77%	ND	1.000	0.9999
Exposed to UV light of 200W h m^{-2} and visible light of 200Klux	0.43%	ND	1.000	0.9999
Dry heated for about 12h at 105 °C	0.43%	ND	1.000	0.9999

ND=not detected

for assay values were found to be 99.9 and 0.5%, respectively. The results indicated the good precision of the developed method.

Accuracy

Accuracy was determined by applying the developed method to synthetic mixtures of excipients to which known amounts of each drug corresponding to 50, 80,100, 120 and 150% of label claim had been added. The accuracy was then calculated as the percentage of analyte recovered from the formulation matrix. Mean recoveries (Mean ± S.D) for EFZ from the formulation are 99.28 ± 1.3%. The inter day accuracy was also determined by assaying the tablets in triplicate per day for consecutive 3 days. Mean recoveries for the interday accuracy was 99.54 ± 1.8%. The obtained result suggested the accuracy of the developed method for the determination of the EFZ in the formulation.

Table 2. Results of robustness study.

Parameter	Observed value		
	Variation	Tailing factor	RSD for five injection of standard (%)
Flow rate	0.9 mL^{-1}	1.36	0.27
	1.1 mL^{-1}	1.37	0.14
Column temperature	25°C	1.38	0.12
	35 °C	1.36	0.11
pH (± 0.2 units of the set pH)	5.8	1.38	0.39
	6.2	1.39	0.12
Mobile phase composition	90% organic	1.32	0.19
	110% organic	1.44	0.13

Table 3. Result of EFA in marketed product.

Marketed formulation	Drug	% Amount found ± SD	% RSD
Efavir	Efavirenz-600 mg	99.40 ± 0.53%	0.53%
Sustiva	Efavirenz-600 mg	98.51 ± 0.18%	0.18%

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. One factor at a time was changed to estimate the effect. Thus, five replicate injections of standard solution were injected under each parameter and observed the change on the tailing factor for efavirenz peak and the R.S.D. for peak area of efavirenz. The flow rate of mobile phase was changed by ± 10% that is 0.9 to 1.1 mL min^{-1}.The effect of pH of buffer in mobile phase was studied by varying ± 0.2 pH units. (pH 5.8 and pH 6.2). The effect of column temperature was studied at 25 and 35°C instead of 30°C. The effect of ±10% of the organic phase composition (acetonitrile) in mobile phase was studied by changing buffer and acetonitrile composition by 49.5:50.5 v/v and 60.5:39.5 v/v. Table 2 represents the robustness of the method.

Solution stability and mobile phase stability

The R.S.D. of assay of EFA during solution stability andmobile phase stability experiments was within 1%. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay determination was stable up to 72 h.

Analysis of marketed products: The validated method was applied for the analysis of EFA film coated tablet of strength 600 mg from two different manufacturers. In both cases assay obtained is more than 98% and no interference of impurity peak observed in Efavirenz peak.

Table 3 indicated that the amount of each drug in the tablet samples met with requirements (95 - 105% of the tablet claim).

Conclusion

The present developed method is selective, rapid, precise and accurate. The retention time is of 6.6 minute. Application of this method for the analysis in tablet shows that neither the degradation products nor the excipients, including the preservatives interfere with the analytical determination. This indicates that the proposed method could be used as a stability-indicating method for the determination of Efavirenz either in bulk powder or in pharmaceutical formulations.

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