Stability Study and Densitometric Determination of Efavirenz in Tablet by Normal Phase Thin-Layer Chromatography

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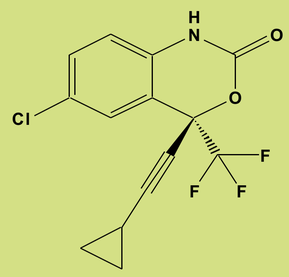
Drug Profile:

Structure:



Category: Antiretroviral

Chemical Name: (4S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoro methyl)-2H-3,1-benzoxazin-2-one

Empirical Formula: $C_{14}H_9ClF_3NO_2$

Molecular Weight: 315.7

Dissociation constant: 9.1

Solubility: Methanol

Method Reported: Determination of Efavirenz by Capillary Electrophoresis[1], MEKC[2], HPLC[3,4], LCMS[5,6], GCMS[7] and HPTLC[8]

Experimental:

Instruments: CAMAG LINOMAT-IV sample applicator with CAMAG TLC SCANNER III (Densitometer) with winCAT'S 4.0 version software

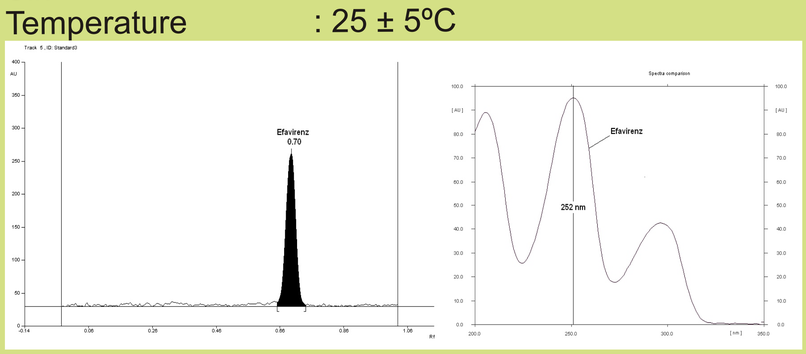
Reagents and Chemicals:

	Drug/ Dosage form/ Chemical	Manufacturer
Pure Drug Sample	Efavirenz (EFA)	Matrix Laboratories Ltd.
Tablet Formulation	*Efferven*	Ranbaxy Laboratories Ltd.
Chemicals	Chloroform, Methanol, Toluene	Qualigens
TLC Plate	Pre-coated silica gel G60, F_{254} HPTLC plates	E-Merck

Standard Solutions: 100 µg/mL of EFA in methanol

Chromatographic conditions:

Mobile phase : Chloroform: Methanol: Toluene [7:1:2 (v/v)]
Scanning wavelength: 252 nm
Stationary Phase : Aluminium precoated TLC plates Silica Gel G60, F254 TLC Plate, size 10 x 10 cm, 200 µm layer thickness
Mode of Application : Band
Band Width : 4 mm
Sample volume : 8 µL
Application rate : 5 sec/µL
Separation technique : Ascending
Development Chamber : Twin trough glass chamber, 10 x 10 cm.
Saturation Time : 15 min with mobile phase and spotted plate
Migration Distance : 80 mm.
Detection : UV Densitometric scanning
Scanning Mode : Absorbance/ Reflectance
Scanning speed : 20 mm/sec
Slit Dimension : 3 × 0.45 mm
Temperature : 25 ± 5ºC



Force degradation studies of EFA:

The stress studies were initiated by using 1 mg/ml solution of EFA (API and Efferven tablet) and exposing it to various stress conditions as follows,

1. **Hydrolytic Degradation:**
   Acidic: 0.1 to 5 N methanolic HCl
   Basic: 0.1 to 5 N methanolic NaOH
   Neutral: Methanolic water
2. **Oxidative Degradation:** 3% H_2O_2 for 7 days
3. **Photolytic Degradation:** Exposing to sunlight for 60 days
4. **Thermolytic Degradation:** Exposing at 70° C for 60 days

Table 1: Total exposure and duration of forced degradation conditions

Stress conditions	Duration of exposure
Acid (2N HCl)	5h reflux
Base (0.1N NaOH)	1h reflux
Neutral (Water)	8h reflux
Oxidative (3% H_2O_2)	7 days at R.T.
Thermal (70°C)	2 month
Photo (Sunlight)	15 days

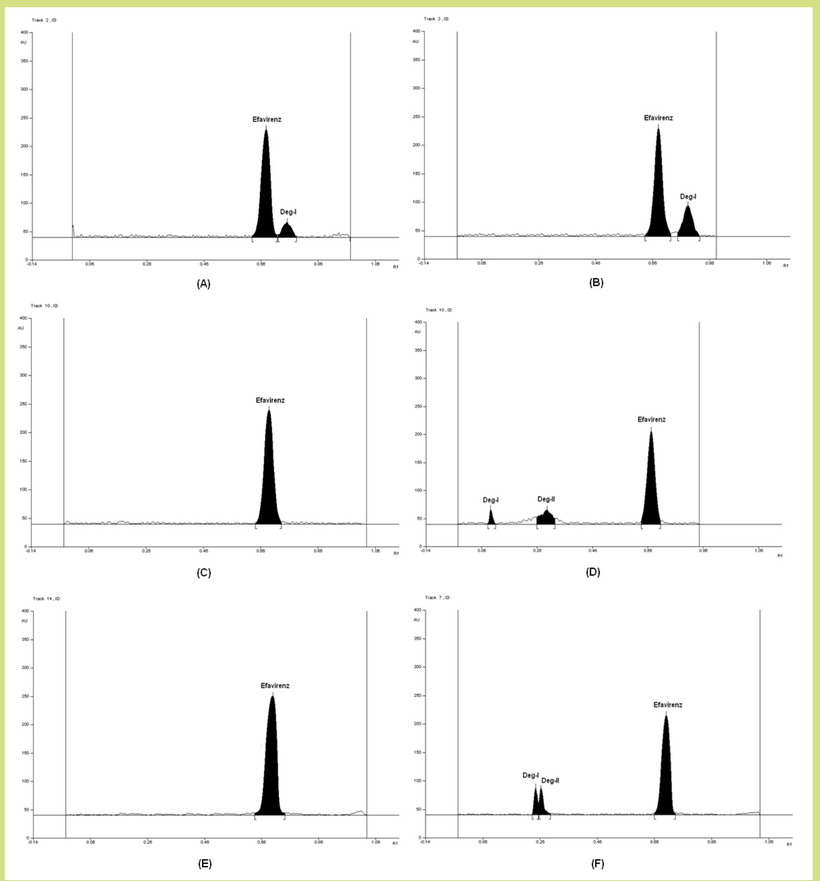
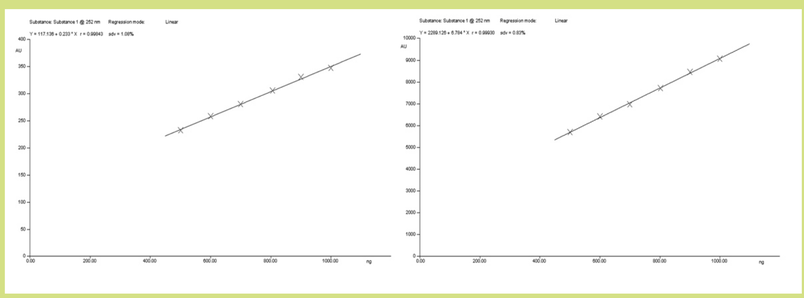


Figure: HPTLC densitograms of forced degraded samples of EFA (A) 2N HCl reflux (5h), (B) 0.1N NaOH reflux (1h), (C) Neutral reflux (8h), (D) 3% H2O2 (7days), (E) Thermal (dry heat) (30 days at 70°C) and (F) Sunlight (15 days)

Preparation of calibration curve:

Aliquot portions of working standard solution (3-14 µl) were applied on the TLC plate and densitograms were developed under optimized chromatographic conditions and the calibration curve was obtained. The curves were found to be linear between concentration range 500-1000 ng/spot both by height and area.



Application of Proposed Method for Estimation in Marketed Formulation:

Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 50.0 mg of EFA (134.46 mg) was transferred into a 50 mL volumetric flask containing little methanol. The powder dissolved in 30 mL methanol and the solution was sonicated for 15 min. The solution was cooled to room temperature and diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. Five milliliters of filtrate was transferred to a 50 mL volumetric flask and then volume was made up to the mark with methanol to obtain a concentration of 100 µg/mL working sample.
Two bands of standard solution and six bands of sample of equal volume (8 µL) were applied on TLC plate and the plate was developed and scanned as per optimized chromatographic conditions.

$$\%\ \text{Labelled claim} = \frac{Ew \times D \times Avg.Wt.}{Va \times Ws \times Lc} \times 100$$

Ew = Drug estimated in applied volume (µL), D = Dilution factor
Va = Volume of sample applied, Ws= Weight of sample
Lc = Labelled claim of drug (mg/ml)

Table 1. Results of HPTLC Assay Studies

EFA	Label claim (mg)	% of labeled claim* ± SD	% RSD
By height	200	99.38 ± 0.4290	0.4506
By area	200	99.69 ± 0.4506	0.4520

*Each value is a mean of five determinations

Validation of proposed method:

Precision:

Formulation			System Precision*	Method Precision*	Intermediate Precision*		
					Interday	Intraday	Different Analysts
EFFERVEN	By height	Mean	99.88	99.31	99.70	99.77	99.48
		SD	1.0753	0.8937	1.1874	0.6834	1.1746
		% RSD	1.0766	0.9000	1.1909	0.6849	1.1808
	By area	Mean	99.88	99.24	99.23	99.54	99.27
		SD	1.1767	0.9392	1.2875	0.6352	0.9868
		% RSD	1.1781	0.9464	1.2975	0.6381	0.9941

*Each value is a mean of six determinations

Accuracy:

EFFERVEN Tablet (Avg. wt. 537.82 mg)						
Sr. No.	% Spiking Level	Wt. of sample + std. EFA# (mg)	Amount of std. drug recovered by area (mg)*		% Recovery*	
			By height	By area	By height	By area
1	80	94.54 + 5.0	5.02	5.11	100.40	102.10
2	100	94.63 + 15.0	14.75	14.90	98.33	99.32
3	120	93.94 + 25.0	24.82	24.81	99.29	99.24
				Mean	99.34	100.22
				SD	1.0343	1.6295
				% RSD	1.0411	1.6260

*Each value is a mean of five determinations,
#Added in the form of standard stock solution

Specificity:

Sr. No.	Sample	% Labeled claim by area	
		By height	By area
1.	Normal	99.07	99.73
2.	Acid	99.12	99.65
3.	Alkali	93.63	94.31
4.	Oxide	86. 32	87.53
5.	Heat	99.17	98.91
6.	Sunlight	99.63	99.96

Ruggedness:

As per precision studies.

Robustness:

Method Parameter		By height			By area		
		Mean*	SD	%RSD	Mean*	SD	%RSD
Wavelength	250 nm	98.89	0.4537	0.4588	99.60	0.9943	0.9983
	254 nm	98.86	0.5805	0.5872	98.29	0.4837	0.4922
Temperature	22°C	98.97	0.6062	0.6125	99.09	0.9466	0.9552
	28°C	99.54	0.5261	0.5285	99.22	1.1847	1.1940
Saturation period	8 min	99.15	0.5399	0.5445	98.85	0.8361	0.8458
	12 min	98.34	0.6606	0.6718	98.80	1.2383	1.2533

LOD & LOQ:

Parameters	By height	By area
Linear dynamic range (ng/band)	500–1000	500–1000
Slope	0.233	6.784
Y-intercept	117.136	2289.126
Correlation coefficient (r)	0.998	0.999
LOD (µg/mL)	164.16	138.45
LOQ (µg/mL)	497.45	419.55

Results and Conclusion:

Results of estimation of marketed formulation of EFA was found to be 99.38±0.4317 and 99.69±0.4506 by height and area respectively.
The average recovery values are obtained were 99.34±1.0343 and 100.22±1.6295.
The proposed method is simple fast cost effective and therefore can be applied for routine quality control of pharmaceutical preparations.

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