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DETERMINATION OF EFAVIRENZ IN TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A simple and cost effective, fast and precise reverse phase high performance liquid chromatographic method is described for the determination of efavirenz in pure form and in pharmaceutical formulations. This method is based on using a Luna 5 µ C_{18} column, the size of coloum 5 micron, 250 x 4.60 mm from phenomenex. The mobile phase is acetonitrile: water (0.02 M sodium dihydrogen orthophosphaste). The ratio of mobile phase is 70 : 30 v/v. the flow rate is 1 mL/min, and effluent was monitored at 270 nm. The elution time was 4.5 min. The linearity range was 10-60 µg/mL for efavirenz.

Key words: Efavirenz, RP- HPLC, C_{18}, Tablet.

INTRODUCTION

Chemically, efavirenz (EFA) is (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-1-benzoxazin-2-one. EFA is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as a part of highly active anti-retroviral therapy for the treatment of a human immunodeficiency virus (Fig. 1)[1]. The drug is used in combination with other anti-retroviral agents for the treatment of HIV-1 infection in children and adults[2]. The usual dose of EFA is 600 mg per day (usually given at bed time). Several methods have been reported for determination of efavirenz achieved separation of alkynes by reversed phase HPLC using ruthenium complexes[3]. The separation of efavirenz in human plasma by using reversed phase HPLC technique using C18 column has also been achieved[4,5]. So far in our knowledge, only one stability indicating method has been reported using cyano column for the determination of efavirenz[6]. The disadvantage of the method is that its run time is about 15 min and gradient separation. The Indian pharmacopoeia[7] reported methods involved complicated time-consuming multi-step liquid-liquid extraction techniques. To the

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best of our knowledge, there is no work reported in the literature about the estimation of efavirenz from pharmaceutical formulation by using RP-HPLC. The purpose of this investigation was to development of a rapid, sensitive and validated HPLC method for quantification of efavirenz from tablets forms.

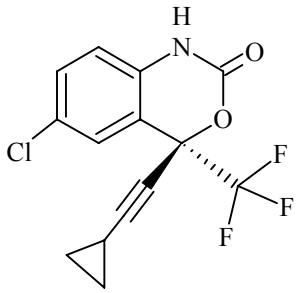

Fig. 1: Chemical structures of efavirenz

EXPERIMENTAL

Materials and method

Standard efavirenz was obtained from Aurobindo Pharma, Hyderabad. Potassium dihydrogen phosphate AR, sodium dihydrogen orthophosphaste AR grade and methanol of HPLC grades were supplied by S.D Fine Chemicals, Mumbai. Water HPLC grade was obtained from a milli-QRO water purification system.

A gradient high-pressure liquid chromatography (Shimadzu HPLC Prominence UFLC Series) with LC-20AT Prominence Pumps, variable wavelength programmable UV/Vis SPD-20A Prominence Detector, SIL-20 AC HT/ Prominence UFLC auto Sampler (Shimadzu) and operating software LC Lab Solution were used. The method was carried on a Phenomenex Luna 5 µ C_{18} (250 x 4.60 mm i.d, 5 µ) column as a stationary phase. The mobile phase consisted of 0.02 M potassium dihydrogen phosphate salt as aqueous phase and acetonitrile. The mobile phase was filtered through a 0.45 µ membrane filter and degassed before analysis. Acetonitrile and aqueous phase was in the ratio of 70 : 30 v/v as the mobile phase and flow rate was 1 mL/min. A SIL-20 AC HT/ Prominence UFLC auto Sampler was used for the injection of sample. Detection was done at 270 nm and separation was carried out at the room temperature of about 20^{o}C.

Standard stock solution of the drug was prepared by dissolving 25 mg of efavirenz in a mixture of methanol : water (1 : 1 v/v) and made up to with 25 mL with the same (1000 µg/mL). Working standard solution was prepared by diluting 1 mL of the stock solution to

10 mL with methanol : water (1 : 1 v/v) (100 µg/mL). The gradient dilution were prepared by taking 1, 2, 3, 4, 5 and 6 mL of solution and made up to 10 mL with methanol : water (1 : 1 v/v) solution. Twenty µL of the solution from each flask was used for experiment. Calibration curve was constructed by plotting mean peak area against the corresponding drug concentration (Fig. 2). The detector response was found to be linear in the concentration range of 10-60 µg/mL (Table 1). The typical chromatogram of efavirenz drug solution is shown in Fig. 3. Calibration curves could be represented by the following equation y = 46135x-32099 (R^2 = 0.9972). This equation was used for the determination of efavirenz from tablets.

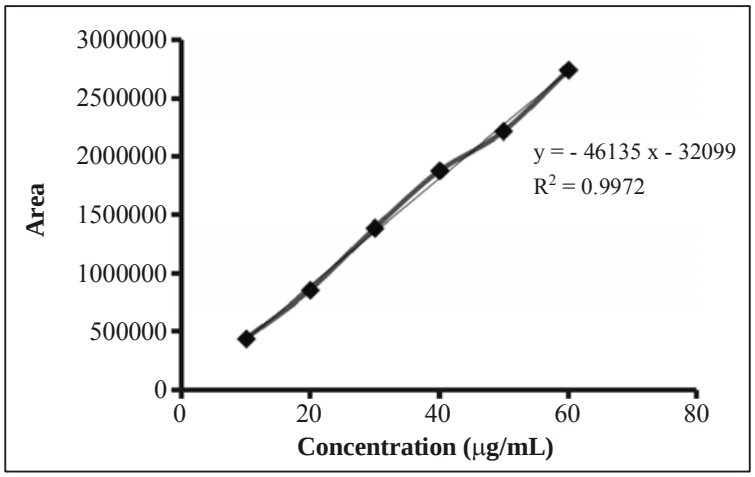

Fig. 2: Efavirenz standard plot (Concentration vs area)

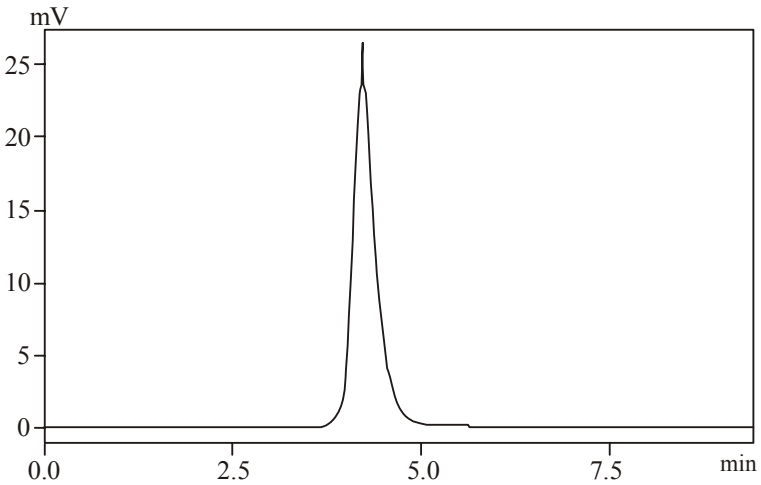

Fig. 3: Chromatogram of efavirenz drug solution

Table 1: Detector response (Concentration vs area)

Concentration (µg/mL)	1	2	3	Average area
10	431309	431937	432003	431750
20	851671	847982	844294	847982
30	1385188	1384692	1378806	1382895
40	1874268	1877546	1877565	1876460
50	2213812	2216498	2209889	2213400
60	2749339	2736797	2743524	2743220

For the estimation of drug from commercial formulation, 20 tablets of two brands-Efavir tablets (Cipla Limited, Mumbai.) and Efavirenz tablets (Strides Arcolab Limited, Bangalore) were used. Each contained 600 mg and 200 mg respectively of efavirenz, were powdered finely. A quantity equivalent to 25 mg was transferred into 25 mL volumetric flask, dissolved and made up to with acetonitrile : water (1 : 1 v/v) solution. The solution was filtered through a 0.45 µ membrane filter. One milliliter of the resulting solution was then diluted to 10 mL with an above used solution. From this, 0.5 and 1 mL sample were taken and their volume was made up to 10 mL each.

RESULTS AND DISCUSSION

A chromatogram of these solutions was obtained by injecting 20 µL of each sample in to the chromatographic system (Fig. 4). There was no interference from diluents and lubricants. The retention time of the drug was 4.5 min. Chromatographic parameters such as peak asymmetry (A) and capacity factor (k) were found to be 1.09 and 0.75, respectively. To study the accuracy, reproducibility, precision of the proposed method; the recovery experiments were carried out. A fixed amount of the pre-analyzed sample was taken and standards were added at three different levels. Each level was repeated five times. The summaries of recovery studies are reported in Table 2.

The present study comprises a high performance liquid chromatography method to determine efavirenz from tablet dosage form. Experiment was carried out to establish the method. The mobile phase, bearing acetonitrile : buffer in proportion of (70:30) was found to be idale. The retention time of efavirenz were found 4.5 min. The value of percent recovery and standard deviation indicate that method is accurate, reproducible and precise. The summaries of final results are illustrated in Table 3.

Table 2: Summary of recovery studies

Drug efavirenz	Label claim tablet (mg)	Amount found (mg)	Recovery studies		
			Amount added (mg/mL)	Amount recovery (mg/mL)	Percentage recovery (%)
Tablet A	10	9.95 ± 0.025	5	14.9 ± 0.05	99.6
			10	20.5 ± 0.11	101.5
Tablet B	10	9.98 ± 0.025	5	15.1 ± 0.11	100.6
			10	19.7.2 ± 0.11	99.8
Tablet-A is Efavir tablets (Cipla Limited, Mumbai.) and Tablet B is Strides Efavirenz tablets (Arcolab Limited, Bangalore)					

Table 3: Summaries of final result

Brand name	Amount found (mg/tablet)	% RSD	Percentage assay
Efavir tablets (Cipla Limited, Mumbai)	10.01 ± 0.025	0.249	100.1
Efavirenz tablets (Strides Arco lab Limited, Bangalore)	9.92 ± 0.025	0.231	99.2

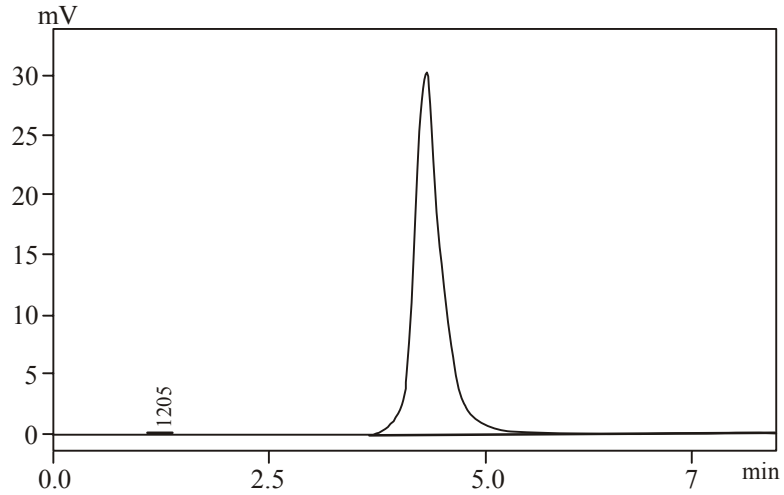

Fig. 4: Chromatogram of the sample tablet solution

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