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Method Development and Validation of Efavirenz in Bulk and Pharmaceutical Dosage Form by Uv Spectrophotometer

B. Prathap*[1], Akalanka Dey[2], G. Srinivasa Rao[1], S.Vennela[1], and Shahul Hussain[1]

[1]Department of Pharmaceutical Analysis, Saastra College of Pharmaceutical Education & Research, Nellore, Andhra Pradesh, India-524311.

[2]Faculty of Pharmacy, Annamali University, Annamalai Nagar, Tamilnadu, India - 608002.

ABSTRACT

A simple, precise, accurate, sensitive and highly selective UV-Spectrophotometric method has been developed and validated for estimation of Efavirenz in bulk and pharmaceutical dosage form. It shows maximum absorbance at 280 nm with methanol and water (60:40). Estimation was carried out by A(1%1cm) and by comparison with standard. Calibration graph was found to be linear (r^2 =0.9983) over concentration range of 10-50µg/ml. The proposed methods appear to be simple, sensitive, and reproducible when checked for parameters like accuracy, precision, limit of detection for routine determination of Efavirenz in bulk as well as in tablet. The methods can be adopted in its routine analysis.

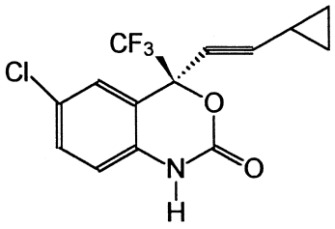
Keywords: Efavirenz, UV- Spectrophotometer, ICH Validation.

INTRODUCTION

Efavirenz (EFV, brand names Sustiva and Stocrin) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active anti retroviral therapy (HAART) for the treatment of a human immune deficiency virus (HIV) type 1. Efavirenz is chemically described as (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one. Its empirical formula is $C_{14}H_9C_lF_3NO_2$. (en.wikipedia.org). It has the structural formula shown in (Figure.1). Efavirenz is a white crystalline powder with a molecular mass of 315.68 g/mol. It is practically insoluble in water (<10 µg/mL) (Anonymous 1).

For HIV infection that has not previously been treated, the United States Department of Health and Human Services Panel on Antiretroviral Guidelines currently recommends the use of efavirenz in combination with lamivudine/zidovudine (Combivir) or tenofovir/ emtricitabine (Truvada) as the preferred NNRTI-based regimens in adults and adolescents. Efavirenz is also used in combination with other antiretroviral agents as part of an expanded post exposure prophylaxis regimen to reduce the risk of HIV infection in people exposed to a significant risk (e.g. needle stick injuries, certain types of unprotected sex etc.) (en.wikipedia.org). The usual adult dose is 600 mg once a day. It is usually taken on an empty stomach at bedtime to reduce neurological and psychiatric adverse effects. Efavirenz was combined with the popular HIV medication Truvada, which consists of tenofovir and emtricitabine, all of which are reverse transcriptase inhibitors. This combination of three medications approved by the U.S. Food and Drug Administration (FDA) in July 2006 under the brand name Atripla, provides HAART in a single tablet taken once a day. It results in a simplified drug regimen for many patients Efavirenz is used to treat HIV infection. It is never used alone and is always given in combination with other drugs.

Fig.1 Structure of Efavirenz



The decision on when to start treatment should take into account CD4 count, HIV viral load, treatment history, resistance profiles and patient preference. Since the preliminary publication of the results of the ACTG

***Corrosponding author**

B. Prathap
Email id: prathapnila@gmail.com

5142 trial in 2006 which compared efavirenz against lopinavir, efavirenz has been used as first line treatment in preference to the protease inhibitors. The ACTG 5095 trial showed that the potency of efavirenz is maintained at all CD4 counts and HIV viral loads (Anonymous 1). Efavirenz is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class (Ren J *et al*., 2002).

As most NNRTIs bind within the same pocket, viral strains which are resistant to efavirenz are usually also resistant to the other NNRTIs, nevirapine and delavirdine. The most common mutation observed after efavirenz treatment is K103N, which is also observed with other NNRTIs (product information, April 2005), Psychiatric symptoms, including insomnia, nightmares, confusion, memory loss, and depression, are common (Cespedes *et al*., 2006) and more serious symptoms such as psychosis may occur in patients with compromised liver or kidney function (Hasse *et al*., 2005 and Lowenhaupt *et al*., 2007), rash, nausea, dizziness and headache may occur. A general guideline about efavirenz and pregnancy states that efavirenz can cause birth defects and should not be used in women who might become pregnant (DHHS Panel *et al*., 2006).

A later study, however, found no increased risk of overall birth defects among women exposed to efavirenz during the first trimester of pregnancy compared with exposure to other antiretroviral drugs (Ford N *et al*., 2010). Safety in children has not been established Use of efavirenz can produce a false positive result in some urine tests for marijuana (Rossai S *et al*., 2006; Roder *et al*., 2007). Abuse of efavirenz by crushing and smoking the tablets for supposed hallucinogenic and dissociative effects has been reported in South Africa, where it is used in a mixture known as whoonga (BBC News, 2008, ABC News, 2009) .

As with most HIV treatments, efavirenz is quite expensive. A one month supply of 600 mg tablets costs approximately $550 in April 2008 (drug store.com, 2008). Some emerging countries have opted to purchase Indian generic (India daily) such as Efavir by Cipla Ltd (Anonymous 2). 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 immune deficiency virus (Sutterlin S *et al*., 2010).

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 give nat 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 (Agnes IV 2008).

Gita *et al*., (2008) and Agnes *et al*., (2008) reported separation of efavirenz in human plasma by using reversed phase HPLC technique using C18column. So far in our knowledge only one stability (Careri M, 1993; Gita R, 2008) 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 runtime is about 15 min and gradient separation (Indian Pharmacopoeia, 2007 and The Merck Index). The Indian pharmacopoeia (Indian Pharmacopoeia, 2007) lso published isocratic HPLC method for the assay of EFA. The Run time is about 15 min. (Montgomery ER, 2001 and Martindale and Holler Skoog).

The present work describes a simple, economical, accurate and reproducible spectrophotometric method for estimation of efavirenz in pharmaceutical formulations. The proposed method was successfully applied for determination of efavirenz in its pharmaceutical formulations.

EXPERIMENTAL

Apparatus

The spectrophotometric measurements were carried out using a Analytical double beam UV-Visible Spectrophotometer model 2060 plus with 1cm matched quartz cell.

Materials

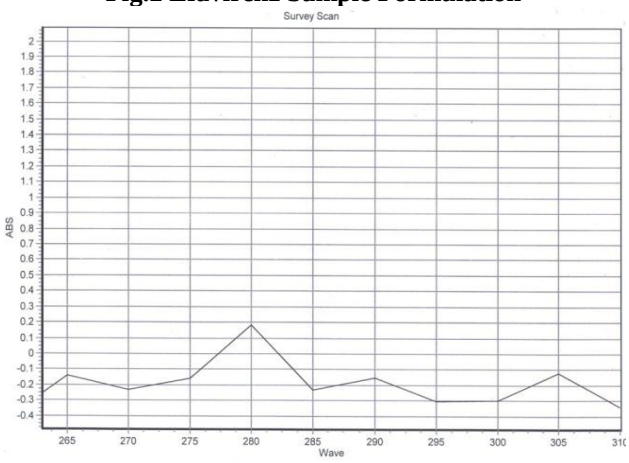
Efavirenz was procured from Matrix Laboratories, India. Methanol (AR grade): double distilled water (60:40) used as solvents throughout the experimentation. Pharmaceutical preparation was purchased from local pharmacy.

Methods

Preparation of working standard drug solution

100 mg standard Efavirenz [EFV] was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol and water (60:40). The flask was shaken and volume was made up to the mark with methanol and water (60:40) to give a solution of 1000 µg/ml. From this solution, 10 ml solution was pipetted out and placed into 100 ml volumetric flask. The volume was made up to mark with methanol and water to give a solution containing 100 µg/ml. All measurements were made at room temperature. The standard solutions were prepared by the proper dilutions of the stock standard solution with distilled water to reach concentration range of 10-50 µg mL-1. The determination was conducted in triplicate.

Fig.2 Efavirenz Sample Formulation



Analysis of marketed formulations

One brand of tablet (Estiva Hetero 600mg) was used for all analytical study. Twenty tablets each containing 600 mg of EFV was weighed. The powder equivalent to 100 mg of EFV was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 50 ml of the methanol and water (60:40) and sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol and water (60:40). The above solution was carefully filtered through Whatmann filter paper (No. 41). From this solution, 10 ml was taken and diluted to 100 ml with methanol and water (60:40) to give a solution of 100 µg/ml.

Preparation of Calibration curve

The standard stock solution of EFV was scanned in the wavelength range of 200 nm to 400 nm against solvent solution as a blank. A calibration curve was constructed over a concentration range 10-50 µg mL-1. Absorbance of each solution was measured at the wavelength of 280 nm. Calibration curve was constructed for efavirenz by plotting absorbance versus concentration at 280 nm wavelength. The determination was conducted in triplicate**.**

Validation of Spectrophotometric method

For UV-Spectroscopy method the following validation parameters were studied.

1) Linearity,
2) Precision,
3) Accuracy,
4) Ruggedness,
5) Limit of Detection,
6) Limit of Quantification.

RESULT AND DISCUSSION

Selection of analytical wavelength

Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200 - 400 nm. Absorption spectra obtained at 280nm. This wavelength, 280 nm was selected as wavelength of analytical measurement for this method (Figure.2).

Fig.3 Calibration Curve of Efavirenz

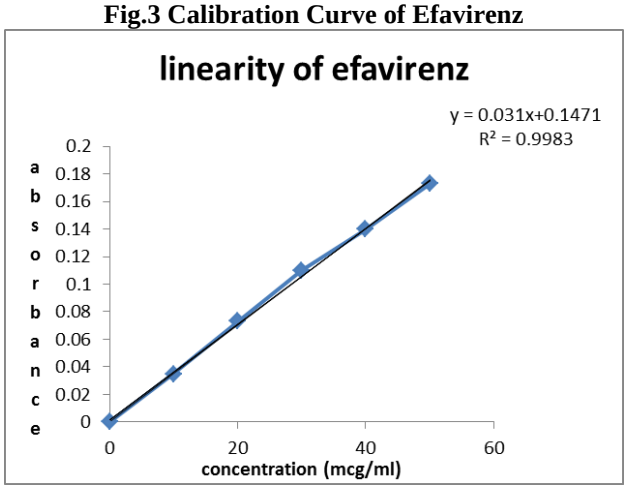


Table.1 Optical Characteristics of EFV

Parameters	Values
λmax (nm)	280nm
Beers Law Limit (µg/ml)	10-50
Regression Equation	Y=0.031X+0.1471
Slope	0.031
Intercept	0.1471
Correlation coefficient (R^2)	0.9983
Limit of Detection (µg/ml)	0.277
Limit of Quantification (µg/ml)	0.84

Table.2 Assay of Sample Formulation

Sample	Label Claim(mg/tab)	Amount Found (mg/tab)	% Label Claim	S.D	%R.S.D
EFV	600	589.09	98.18	0.021	0.42

Table.3 Determination of Accuracy results of EFV

Levels	Amount of Sample (µg/ml)	Amount of Drug added (µg/ml)	Amount recovered*	% Recovery
50%	50	50	99.37	99.37
100%	50	100	149.79	99.86
150%	50	150	201.86	100.93

*Average of six determinations

Table.4 Determination of Precision results for EFV at 280nm

Label Claim (mg/tab)	Intraday			Interday		
	Amount found*	% Label Claim	% R.S.D	Amount Found*	% Label Claim	% R.S.D
600	598.51	99.75	0.92	606.7	101.12	0.76

*Average of six determinations
R.S.D – Relative Standard Deviation

Table.5 Ruggedness results for EFV at 280nm

Sample	Label Claim (mg/tab)	Analyst I		Analyst II	
		Amount found*	% Recovery	Amount found*	% Recovery
EFV	600	589.09	98.18	598.75	99.79

*Average of six determinations

Calibration curve

Appropriate volume of aliquots from standard EFV stock solution II were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol and water (60:40) to obtain concentrations of 10, 20, 30, 40 and 50 µg/ml. Absorbance of each solution against methanol and water (60:40) as blank were measured at 285 nm and the graph of absorbance against concentration was plotted and is shown in Figure.3. The regression equation and correlation coefficient were determined which are presented in Table.1. Determination of Sample formulation was presented in Table.2.

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures (Validation of analytical Procedure Q2 (A) 1994, Q2 (B), 1996, Q2(R1), 1996).

Efavirenz has the absorbance maxima at 280 nm for the UV-Spectroscopy method. The optical characteristics such as Beer's law limits, Limit of detection and Limit of quantification etc., in each method were calculated and the results were presented in Table.1 respectively. Also the regression characteristics like slope (b), intercept (a), and correlation coefficient (R^2) using the method of least squares were calculated and were presented in Table.1 respectively. The results showed that the methods have reasonable precise.

The validation parameters like Precision, Accuracy, and Ruggedness results were presented in Table.3, 4, 5 and 6 for UV-Spectroscopy method. There was no any interference of excipents in recovery study of the method. From the results these methods was applicable for both bulk and pharmaceutical dosage for the estimation of Efavirenz.

CONCLUSION

The developed and validated UV-Specctrophotometry method reported here is rapid, simple, accurate, sensitive, and specific. This method was validated as per ICH guidelines and results of accuracy, precision, ruggedness was in the limit. There was no any interference of excipients in the recovery study. The method was also successfully used for quantitative estimation and analysis of efavirenz from formulation. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of efavirenz from bulk drug and formulations.

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