IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 1 Ser. II (Jan –Feb 2020), PP 15-18
www.Iosrjournals.Org

Method Development and Validation of Efavirenz by UV Spectrophotometer

B.V. Narasimha Rao[1], N. Sunitha[*1], E. Prabhu Teja[2], G.Salman Raju[2], K. Ananda Babu[2], K.V. Saibhaskar[2], P. Pravallika[2], B.Aksa[2].

1. Chalapathi Institute of Pharmaceutical Sciences, Lam , Guntur
2. Victoria College of Pharmacy, Nallapadu, Guntur
Corresponding author: N. Sunitha Assistant Professor Department of Pharmaceutical Analysis Chalapathi Institute of Pharmaceutical Sciences LAM, Guntur -522006

Abstract: *The developed method for Efavirenz determined in tablet was found to be simple, sensitive, precise, selective, rapid and economic. Efavirenz exhibited maximum absorption at 291nm and obeyed Beer's law in the concentration range of 10-50µg/ml, showed linear regression y=0.181x+1.071 with correlation coefficient (r^2) of 0.999. Recoveries obtained do not differ significantly from 100% showed that there was no interference from the common excipients used in tablet formulation indicating accuracy and reliability of the method. The proposed method can be used for drug analysis in routine quality control & method proves to be more economical than the other standard methods. The developed and validated UV spectrophotometry method 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 in quantitative estimation and analysis of Efavirenz from formulation.*

Key Words: *Efavirenz, Accuracy, Spectrophotometer, Quality Control.*

Date of Submission: 17-01-2020 Date of Acceptance: 05-02-2020

I. Introduction

Efavirenz, a non-nucleotide reverse transcriptase inhibitor is used in highly active human immune deficiency virus type1. It is a white crystalline powder. Efavirenz is also used in combination with other antiretroviral agents as part of an expanded post exposure prophylaxisregimen to reduce the risk of HIV infection.The usual adult dose of Efavirenz is 600mg once a day. Efavirenz inhibits the activity of viral RNA-directed DNA polymerase. Antiviral activity of efavirenz is dependent on intracellular conversion to the active phosphorylated form. Thus, reverse transcriptase inhibitors are virustatic and do not eliminate HIV from the body.Even though human DNA polymeraseis less susceptible to the pharmacologic effects of triphosphorylated efavirenz,this action may never account for some of the drug toxicity.

The UV- Spectrophotometric method for the simultaneous determination of Efavirenz and combination of few drugs were carried out with acetonitrile: water (50:50, v/v) solvent system. Linearity was observed over a range of 1-20µg/mL for Efavirenz, 1-10µg/mL . In another method a quantitative estimation of Efavirenz in bulk and tablets was described, in methanol: water (80:20, v/v), Efavirenz exhibits an absorption maximum at 245 nm and method obeys Beer's law.

EXPERIMENTAL SECTION

METHOD DEVELOPMENT

The develop simple, sensitive, accurate, precise, reproducible, rugged, and robust and relatively inexpensive analytical method (UV- Spectrophotometric) for the analysis of Efavirenz.

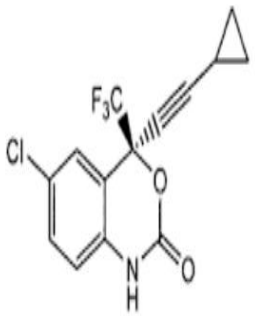
Instrument used UV-Visible spectrophotometer

DRUG PROFILE

DRUG NAME : Efavirenz

IUPAC NAME : (S)-6-Chloro-4-(cyclopropylethynyl) -1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one

STRUCTURE :



MOLECULAR FORMULA : $C_{14}H_9ClF_3NO_2$
MOLECULAR WEIGHT : 315.675g/mol
MELTING POINT : 139-141^{0}C
Pka : 10.2Pka
DESCRIPTION :
Efavirenz is a non-nucleotide reverse transcriptase inhibitor and is used a part of highly active human immune deficiency virus type1. It is a white crystalline powder. The usual adult dose of efavirenz is 600mg once a day.

MECHANISM OF ACTION:

Efavirenz inhibits the activity of viral RNA-directed DNA polymerase.antiviral activity of efavirenz is dependent on intracellular conversion to the active phosphorylated form. Inhibition of reverse transcriptase interfers with the generation of DNA copies of viral RNA,which,in turn interfere with the synthesis of new virions. Intracellular enzymes subsequently eliminate the HIV particle that previously been uncoated and left unprotected,during entry into the host cell. Thus, reverse transcriptase inhibitors are virustatic and do not eliminate HIV from the body.

PHARMACOLOGY:

Efavirenz a non-competitive inhibitor of HIV 1 reverse transcriptase (RT). It has no inhibitory effect on HIV-2 RT or human cellular DNA polymerases alpha, beta, gamma, or delta. Efavirenz binds directly to RT and inhibits viral RNA- and DNA-dependent DNA polymerase activities by disrupting the catalytic site. Although the drug RT template complex may continue to bind deoxynucleoside triphosphate and to catalyze its incorporation into the newly forming viral DNA at a slower rate.

USES :
Efavirenz is a non-nucleoside reverse transcriptase inhibitor with activity against HIV. It is used with other anti-retrovirals for combination therapy of HIV infection.

MATERIALS AND METHODS
Instrumentation
Shimadzu, a double-beam spectrophotometer was used for the detection of absorbance, Mettler Toledo, weighing balance and Misonix sonicator were used.

PROCEDURE
Preparation of stock solution

100mg of pure drug Efavirenz weighed and transferred to 100ml 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 solution of 1000µg/ml. From this solution, 10ml solution was pipette out and transferred into 100ml volumetric flask. The volume was made up to the mark with methanol and water to give solution 100µg/ml. The standard dilutions were prepared by proper dilutions of the stock standard solution with methanol and water to reach the concentration range of 10-50µg/ml.

Preparation of sample solution

EFAVIR-600mg was used for the analytical study. The average weight of tablets was determined by weighing 20 tablets, powdered. Tablet powder equivalent to 100mg Efavirenz was accurately weighed and transferred to a volumetric flask. Make the volume of the solution with methanol and water up to the mark, was

scanned in the wavelength of 291nm. A calibration curve was constructed over a range 10-50µg/ml. The calibration curve was constructed for efavirenz at 291nm wavelength.

METHOD OF VALIDATION:

ACCURACY:

The accuracy of the method was established by adding the Efavirenz test standard solution of the tablet formulation, performed in triplicate and the mean recovery of Efavirenz was measured. The percent (%) recovery at each level was found to be well within the range of 96.4% to 99.3% , indicating insignificant interference from the excipients.

Table1: Table showing the accuracy of standard solution

Concentration (µg/ml)	Amount found	% RSD
20	19.9 ±0.03	0.103
40	38.24 ±0.04	0.065
60	58.06± 0.19	1.025

PRECISION:

The precision and were determined with standard quality control samples prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intra- day) and intermediate precision (inter-day) and RSD% for replicate measurements.

RUGGEDNESS:

Ruggedness is estimated for another analyst.

Table 2: Table showing the precision study

	Analyst1		Analyst2	
Drug	%Amount	%RSD	%Amount	%RSD
EFAVIR 600mg	95%	1.155	94.8%	1.911

LINEARITY:

The linearity method is evaluated by least-square regression method

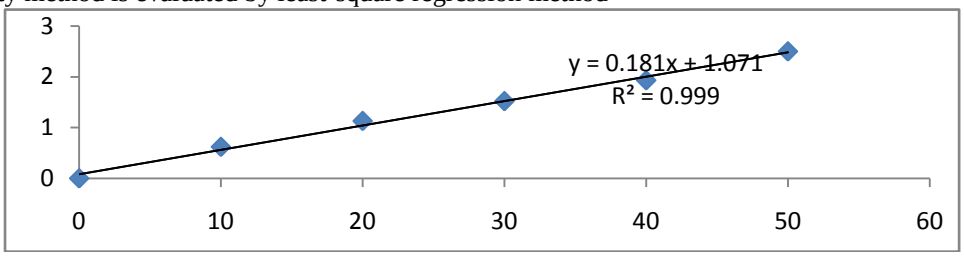


Fig 1: Figure showing the linearity

Table 3: Summary of the Validation Parameters

S.NO.	Parameters		Results
1.	λmax		291nm
2.	Regression line equation		y=0.181x+1.071
3.	Slope		0.181
4.	Intercept		1.071
5.	Correlation coefficient(R)		0.999
6.	Coefficient variation		33.56
7.	precison	Intraday precision	0.06-0.12
		Interday precision	0.065-0.125
8.	Ruggedness	Analyst-1	1.155
		Analyst-2	1.911
9.	LOD		39.74
10.	LOQ		120.44

II. Results And Discussions

The developed method for the determination of Efavirenz in tablet dosage form was found to be simple, sensitive, precise, selective, rapid and economic. Efavirenz exhibited maximum absorption at 291nm and obeyed Beer's law in the concentration range of 10-50 µg/ml. The proposed method for determination of Efavirenz showed linear regression y=0.181x+1.071 with correlation coefficient (r^2) of 0.999. Interday and Intraday studies showed high degree of repeatability of an analytical method under normal operating conditions. The %RSD for precision is less than 2%. There is no interference in tablet formulation.

III. Conclusion

The UV spectrophotometry method is rapid, simple, accurate, sensitive and specific and validated as per the ICH guidelines and found no interference. Thus is considered useful and industrially applicable for quality control.

Bibliography

[1]. Eric Reid, Ian D. Wilson, Methodological Survey in Biochemistry and Analysis Volume20: Analysis for Drug and Metabolites, Including Anti- infective Agents, 1990,1-57.
[2]. Hokanson G.C, A life cycle approach to the validation of analytical methods during pharmaceutical product development, Part II: Changes and the need for additional validation, Pharm.Tech., Oct. 1994, pp. 92–100.
[3]. Renger B, Jehle H, Fischer M and Funk V, Validation of analytical procedures in pharmaceutical analytical chemistry: HPTLC assay of theophylline in an effervescent tablet, J. Planar Chrom. 8:269–278 (July/August 1995).
[4]. Green J.M, A practical guide to analytical method validation, Anal. Chem. News &Features, 1 May 1996, pp. 305A–309A.
[5]. Braggio S., Barnaby R. J., Grosi P, Cugola M., A strategy for validation of bioanalytical methods, Journal of Pharmaceutical and Biomedical Analysis 1996, 14, 375- 388.
[6]. Wegscheider, Validation of analytical methods, in: Accreditation and quality assurance in analytical chemistry, edited by H. Guenzler, Springer Verlag, Berlin (1996).
[7]. Mohammad A., Tabrizi-Fard, Ho-Leung, Fung, Reversed-phase high- performance liquid chromatography method for the analysis of nitro-arginine in rat plasma and urine,Journal of Chromatography B, 679, 1996, 7-12.
[8]. Bmscheck Torsten, Meyer Hartmut, Wellhrner Hans Herbert, a High- performance liquid chromatographic assay for the measurement of azathioprine in human serum samples, Journal of Chromatography B, 675, 1996,287-294
[9]. Causon Roger, Validation of chromatographic methods in biomedical analysis viewpoint and discussion, Journal of Chromatography B, 689 (1997) 175-180.
[10]. Seno S, Ohtake S and Kohno H, Analytical validation in practice at a quality control laboratory in the Japanese pharmaceutical industry, Accred. Qual. Assur. 2:140–145 (1997).

N. Sunitha, et.al. "MethodDevelopment and Validation of Efavirenz by UV Spectrophotometer." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(1), (2020): pp. 15-18.