ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF EFAVIRENZ IN BULK AND ITS CAPSULE DOSAGE FORMS

Bonthu Chandra Priyanka[1], Mandepudi Lakshmi Chandini[1], Thanniru Durga Bhavani[1], Narender Boggula[1], L Satyanarayana[1], Banothu Bhadru[2*]

[1]Omega College of Pharmacy, Edulabad, Ghatkesar, Medchal, Hyderabad, Telangana, India.
[2]CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India.

*Corresponding author E- mail: bhadru.banothu@gmail.com

ARTICLE INFO

Key words:
Efavirenz, antiretroviral, reverse transcriptase inhibitor, capsule dosage form, validation.

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Pharmaceutical analysis is crucial for maintaining bulk drugs quality and safety standards. A simple, specific, sensitive, robust, accurate, precise, and fast UV spectroscopic technique has been devised to estimate efavirenz in bulk and its capsule dosage form quantitatively. The solvent used in this method was 0.5% sodium lauryl sulphate. The wavelength 280 nm was selected to estimate the efavirenz in the capsule dosage form. The linearity ranges between 2-12 µg/ml. The percent recovery was found to be 99.73-100.56%. The correlation coefficient of efavirenz was found to be 0.999. The developed method was simple, specific, sensitive, accurate, precise, rapid, and economical, which can be efficiently and quickly applied to bulk and pharmaceutical dosage forms.

INTRODUCTION:

Efavirenz is used as an antiretroviral (non-nucleoside reverse transcriptase inhibitor) drug (structure shown in Figure 1). It occurs in white to slightly pink crystalline powder. It dissolves well in methanol but is almost insoluble in water [1,2]. The molecular formula of efavirenz is $C_{14}H_9ClF_3NO_2$, and the pKa value is 10.2. IUPAC name of efavirenz is (4S)-6-Chloro-4-(2-cyclopropyl ethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one. Efavirenz should be kept at room temperature, 68°F to 77°F (20°C to 25°C) in a well-closed container and protected from light [3-5]. The aim of the present research is to develop a simple, accurate, precise and a fast UV spectroscopic Technique for determining the quantity of efavirenz in both its bulk and capsule dosage form.

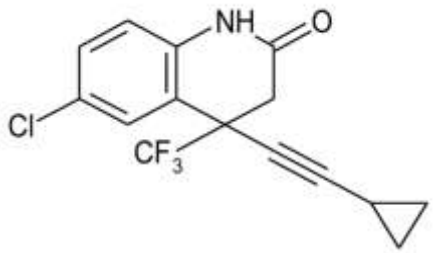

Figure 1: Structure of efavirenz

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents Used: Efavirenz standard drug, efavirenz capsules, HPLC grade methanol & water, sodium lauryl

sulphate (finer), sodium hydroxide, ethyl cellulose, and polyethylene glycol.

2.2 Instruments Used: UV-visible spectrophotometer with PDA detector, electronic balance, Biotechs Ultra sonicator, pH meter, water bath shaker, centrifuge, and refrigerator.

2.3 Preparation of Diluent: Weigh accurately 500mg of sodium lauryl sulphate and transfer into a 100ml volumetric flask, dissolve it with a few mL of distilled water, and finally make the volume [6,7].

2.4 Preparation of Standard: We weighed accurately and transferred about 100mg of efavirenz working standard into a 100ml volumetric flask, added about 80ml of diluent, sonicate to dissolve, and diluted to volume with the same. Filtered the solution through a 0.45µm nylon filter or 0.42µm Whatman filter. The standard preparation is stable for 24 h in the refrigerator.

3. VALIDATION OF PROPOSED METHOD [8-10]

3.1 Linearity- The linearity and range of different concentrations of efavirenz solutions were prepared. The range of the solutions varies from 2-12µg/ml. The absorbance of these solutions is noted. The graph of concentration vs absorbance of linearity solutions was plotted.

3.2 Precision- Precision studies were conducted to verify the reproducibility of the proposed method. In order to evaluate intraday precision, drug concentrations of 10µg/ml of efavirenz were prepared and analyzed six times throughout the day. Interday precision was assessed by preparing drug concentrations of 10µg/ml of efavirenz and analyzing them on three separate days.

3.3 Accuracy- The precision of the suggested approach was assessed through recovery experiments. These experiments involved the addition of varying quantities (80%, 100%, and 120%) of the pure substance to the pre-analyzed mixture. The solutions were made in triplicates, and the percentage of recovery was computed.

3.4 Specificity- The specificity studies were carried out by attempting deliberate degradation of the capsule sample with exposure to stress conditions like acid (5N HCl), alkali (5N NaOH), and oxidizing agent (30% H_2O_2). Specificity studies also include placebo interference and impurity interference.

3.5 Sample solution: The sample solutions were prepared as follows for different stress conditions:

3.5.1 Sample (control): Twenty capsules were accurately weighted, and the average was calculated. An accurately weighed quantity of capsule powder equivalent to about 100mg of efavirenz was transferred to a 100mL volumetric flask, sonicated with 80mL of diluents with intermediate shaking for 15 min. The volume was made up to the mark with diluents. 1mL of the above solution was diluted to 100mL with diluents, and the resulting solution was filtered through a 0.42µm Whatman filter. The final solution containing 10ppm of efavirenz was used as a sample solution.

3.5.1.1 Acid degradation: An accurately weighted quantity of tablet powder equivalent to about 100mg of efavirenz was transferred to a 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min. To this flask, 5mL of 5N HCl was added, and this solution was placed in a water bath at 60°C for two h. Subsequently, the solution was permitted to cool down to room temperature, followed by neutralizing the sample solution with 5mL of 5N NaOH. The volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter. The final solution containing 10ppm of efavirenz was used as a sample solution.

3.5.1.2 Alkali degradation: An accurately weighed quantity of tablet powder equivalent to about 150mg of efavirenz was transferred to a 100 mL volumetric flask, sonicated with

80 mL of diluent with intermediate shaking for 15 min. To this flask, 5mL of 5N NaOH was added, and this solution was placed in a water bath at 60°C for two h. Subsequently, the remedy was permitted to cool down to ambient temperature, and the sample solution was neutralized with 5mL of 5N HCl. The volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100 mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter. The final solution containing 10ppm of efavirenz was used as a sample solution.

3.5.1.3 Peroxide degradation*:* An accurately weighted quantity of tablet powder equivalent to about 100mg of efavirenz was transferred to two 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min.

3.5.1.3.1 Condition 1: To this flask, 5mL of 30% H_2O_2 solution was introduced and subsequently immersed in a water bath set at a temperature of 60°C for a duration of 2 h. Following this, the solution was permitted to cool down to room temperature, and its volume was then adjusted to the desired level by adding diluent. 1mL of the above solution was diluted to 100 mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter. The final solution containing 10ppm of efavirenz was used as a sample solution.

3.5.1.3.2 Condition 2*:* To this flask, 5mL of 30% H_2O_2 was added, and the solution was left at room temperature for 30 min before being adjusted to the desired volume with diluent. 1mL of the above solution was diluted to 100mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter. The final solution containing 10ppm of efavirenz was used as a sample solution.

3.5.2 Placebo solution: Placebo solutions were prepared as follows for different stress conditions:

3.5.2.1 Placebo (control): An accurately weighted quantity of placebo was transferred to a 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min. The volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter.

3.5.2.2 Acid degradation: An accurately weighed quantity of placebo was transferred to a 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min. To this flask, 5mL of 5N HCl was added, and this solution was placed in a water bath at 60°C for 2 h. The solution was then permitted to cool down at ambient temperature, and the sample solution was neutralized with 5mL of 5N NaOH. The volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100 mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter.

3.5.2.3 Alkali degradation: An accurately weighed quantity of placebo was transferred to a 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min. To this flask, 5mL of 5N NaOH was added, and the solution was immersed in a water bath set at 60°C for a duration of 2 h. Subsequently, the solution was left to cool down to room temperature, after which the sample solution was neutralized using 5mL of 5N HCl. The volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100 mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter.

3.5.2.4 Peroxide degradation: An accurately weighted quantity of placebo was transferred to a 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min. To this flask, 5mL of 30% H_2O_2 was added, and this solution was placed in a water bath at 60°C for 2 h. Then, the solution was allowed to cool at room temperature,

and the volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100 mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter.

3.5.3 Stability of Standard and sample solution: Stability refers to the ability of a drug substance or drug product to maintain its identity, strength, quality, and purity within the predetermined specifications throughout the retest or expiration dating period. The primary goal of conducting stability studies is to ascertain the shelf life, which denotes the duration of storage under specific conditions during which the drug product continues to meet its established specifications. Here, we will ensure the stability of the Standard and solutions prepared according to the proposed method.

3.5.3.1 Standard solution: Weighed accurately and transferred about 100mg of efavirenz working standard into a 100ml volumetric flask. Added about 80ml of diluent and sonicate to dissolve and diluted the volume with the same. Further, 1ml of the above solution is diluted to 100ml with diluent and mixed. Filter the solution through 0.42µm Whatman filter. The result is shown in the table.

3.5.3.2 Samples Solution: Twenty capsules were accurately weighted, and the average was calculated. An accurately weighed quantity of capsule powder equivalent to about 100mg of efavirenz was transferred to a 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 15 min. The volume was made up to the mark with diluent. 1mL of the above solution was diluted to 100 mL with diluent, and the resulting solution was filtered through a 0.42µm Whatman filter. The final solution containing 10ppm of efavirenz was used as a sample solution. Two replicate sample solutions were prepared in a similar manner. The Standard and the samples were measured after preparation, and the values were tabulated. After 48 h, a fresh standard was prepared and measured five times to check the system's suitability, and along with the standard, the stability samples, i.e., the standard and the sample solutions, were again measured, and the difference was observed.

4. RESULTS AND DISCUSSION

Optimized spectroscopic conditions: The following parameters were used for spectroscopic analysis of the assay of the pharmaceutical dosage form. 0.5% Sodium lauryl sulphate is used as a solvent, and the maximum wavelength is 280nm (Figure 2).

$$\%\textbf{Assay} = \frac{\text{Sample absorbence}}{\text{Stad.absorbence}} \times \frac{\text{Wt.of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Wt.of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of cap.}}{\text{Label claim}} \times 100$$

4.1 Linearity: The linearity graph was plotted against the absorbance and the concentration, as shown in Figure 3, and the slope was calculated through linear regression utilizing the least squares technique. Table 1 demonstrates the values, revealing a direct correlation between the absorbance and concentrations of efavirenz. The graph clearly showed that the slope line passes through the origin and touches almost all points [different concentration], and the absorbance was measured at a particular wavelength of 280nm in 0.5% SLS (sodium lauryl sulphate). The regression coefficient of the linearity study was found to be 0.999. The slope of the linearity is y = 0.139.

4.2 Precision: The precision study was carried out & the values of individual determination were calculated and presented in Table 2. The precision (repeatability) study shows that there is no significant difference in the precision values; hence, the developed method can be used to analyze the efavirenz in capsule formulation. It was found that there is no evidence of interference between excipients and efavirenz. The analysis of the study lies between 98.33% and 100.56%

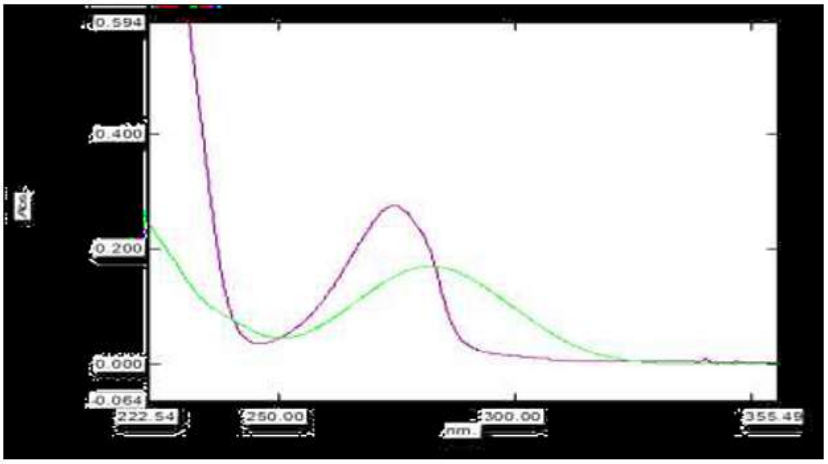

Figure 2: Wavelength maximum of efavirenz

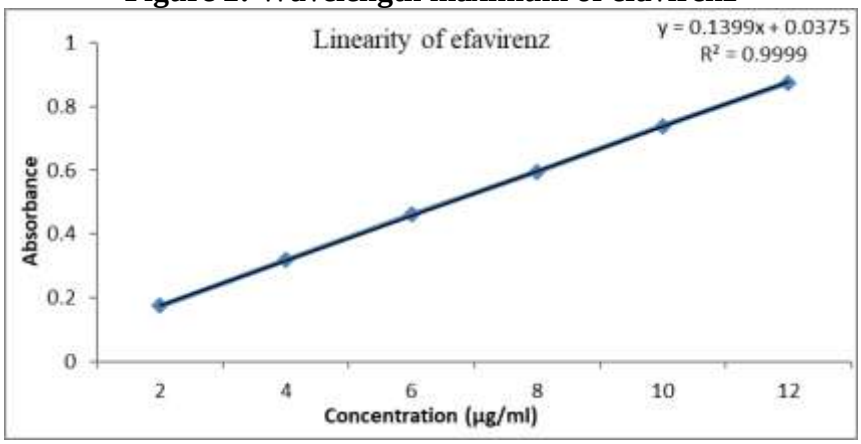

Figure 3: Linearity graph of efavirenz

Table 1: Linearity of efavirenz at 280nm & statistical data of the regression equation

S. No.	Conc. (µg/ml)	Absorbance	Efavirenz	
1	2	0.175		
2	4	0.318	Parameters	280 nm
3	6	0.461	Conc. (µg/ml)	2-12µg/ml
4	8	0.595	Correlation	0.999
5	10	0.739	Slope	0.139
6	12	0.875	Y-intercept	0.037

Table 2: Precision of efavirenz capsule

S. No.	Weight of powder (mg)	Absorbance
1	46.9	0.326
2	46.9	0.324
3	46.9	0.327
4	46.9	0.332
5	46.2	0.336
6	46.9	0.338
	Mean	**0.330**
	SD	**0.005899**
	%RSD	**1.7**

Table 3: Recovery studies of efavirenz

Level (%)	Drug content in mg	Pure drug added in mg	Total mg of content	Absorbance	mg drug recovered	% of drug recovered	Average
50	25.0	12.5	37.5	0.252	36.9	98.5	
50	25.0	12.5	37.5	0.259	38.21	101.5	**99.73%**
50	25.0	12.5	37.5	0.253	37.39	99.2	
100	25.0	25.0	50.0	0.325	49.69	99.00	
100	25.0	25.0	50.0	0.332	50.65	101.0	**98.33%**
100	25.0	25.0	50.0	0.322	49.19	98.0	
150	25.0	37.5	62.5	0.364	64.41	102.0	
150	25.0	37.5	62.5	0.351	60.51	98.4	**100.56%**
150	25.0	37.5	62.5	0.361	63.46	101.3	

Table 4: LOD and LOQ results of efavirenz

Efavirenz	
Slope	0.139
Standard deviation	0.005899
LOD (µg/ml)	0.14
LOQ (µg/ml)	0.42

Table 5: Ruggedness of efavirenz

S. No.	Assay (% of claim) efavirenz	
	Analyst 1	**Analyst 2**
1	99.73	99.88
2	98.55	98.73
3	100.12	100.1
4	99.73	99.63
5	98.93	98.33
Mean	**99.41**	**99.33**
SD	**1.397**	**1.316**
RSD	**1.3**	**1.3**

The specificity and stability studies were calculated and tabulated in Table 6 and 7.

Table 6: Specificity studies of efavirenz

Sample with condition	Sample weight (mg)	Absorbance	Assay of efavirenz		% Degradation
			mg/unit	% of claim	
Sample control	199.89	0.259	59.35	99.6	0.4
With acid	199.79	0.262	56.35	98.2	1.8
With base	199.09	0.255	57.35	95.8	4.2
With H_2O_2	199.90	0.263	59.05	48.7	52.3

Table 7: Stability of efavirenz standard and test solution

	Wt. of std taken	Absorbance of std	Wt. of test taken	Absorbance of test	% Assay
Initial	99.96	0.255	99.95	0.255	100.00
24 h	99.99	0.260	99.97	0.261	100.38
48 h	99.95	0.259	99.98	0.257	98.84

It shows only a 2% deviation, which is allowed in any capsule dosage form. The mean value of the precision study is 99.54%.

4.3 Accuracy (recovery studies) Recovery study for efavirenz was carried out at 50%, 100% & 150% concentration. The results were shown in the Table 3 from the below data drug-drug interaction, drug excipients interaction, and the drug solvent interaction has not been noticed or identified. Hence, there is no interference of any component with the drug has been proved.

4.4 LOD and LOQ

The LOD and LOQ are calculated as;

LOD = 3.3 × (S.D./Slope)

LOQ = 10 × (S.D./Slope)

Where,

SD = Standard deviation of the Y-intercepts of the 6 calibration curves, Slope = Mean slope of the 6 calibration curves. The LOD and LOQ values are calculated and illustrated in Table 4.

4.5 Ruggedness- The ruggedness was determined by using the data obtained by the analysis performed by different analysts using different reagents, different instruments, and different columns. Each analyst prepared six samples of the same batch. The results obtained are given in Table 5. The literature survey revealed that few methods are available for the estimation of the efavirenz dosage form, but a straightforward, cost-effective, and suitable technique is required to determine the aforementioned combination in a unified dosage form. Hence, an attempt has been made to develop the method using the spectroscopic method for the estimation of the efavirenz dosage form. Using 0.5% sodium lauryl sulphate was detected at 280nm.

5. CONCLUSION

The UV method was determined to be linear, precise, reliable, specific, resilient, and cost-effective. The solvent is easy to prepare and cost-effective. The sample recoveries in all formulations aligned well with their respective label claims, indicating no interference from formulation excipients in the estimation process. Additionally, this method offers an advantage over the previously reported method as the absorbance of the drugs is below 1, allowing for a quick assay. As a result, it works well for routine drug analysis using prescription dose forms. The suggested technique may prove beneficial for national quality control laboratories

Ethics approval: Not applicable

Declaration of competing interest: The authors declared that they have no competing interests.

REFERENCES

1. Ravikumar K, Sridhar B, Molecular and Crystal Structure of Efavirenz, a Potent and Specific Inhibitor of HIV-1 Reverse Transcriptase, and Its Monohydrate, Mol Cryst Liq Cryst. 515 (2009) 190–198.
2. Kadam AA, Babu CJG, Permal RV, Channabasavaraj K, Mani TT, Development and Validation of Method for the Quantitative Estimation of Efavirenz in Bulk and Phrmaceutical Dosage Form, Am J PharmTech Res. 2 (2012) 324–330.

3. Shweta Gupta, Kesarla, Narendra , Development and validation of reversed-phase HPLC gradient method for the estimation of efavirenz in plasma, PLoS One. 12 (2017) e0174777.
4. Heydari R, Rashidipour M, Naleini N, Determination of efavirenz in plasma by dispersive liquid-liquid microextraction coupled to high-performance liquid chromatography, Curr Anal Chem. 10 (2014) 280–287.
5. Mahdi A, Hameed DM, Al Hindawi AM, Najm NH, Spectrophotometric Methods for Estimation of Metformine HCl and Sitagliptin Phosphate Drugs in Bulk and Some Pharmaceutical Preparations, International Journal of Drug Delivery Technology. 11 (2021) 347-350.
6. Gouthami Thumma, Narender Boggula, Vasudha B, Anand Kumar Chettupalli, Method Development and Validation for the Simultaneous Analysis of Duloxetine HCL and Methylcobalamine by RP-HPLC, International Journal of Pharmaceutical Quality Assurance. 9 (2018) 363-367.
7. Rebiere , Mazel B, Civade C, Bonnet, Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography, Journal of Chromatography. Analytical Technologies in the Biomedical and Life Sciences. 850 (2007) 376-383.
8. Thaidala Sriveni, Vanamala Naveen, Vemula Sai Rupa, Aeruva Renuka, Sunil Porika, Akiful Haque, Vasudha Bakshi, Narender Boggula, Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy, International Journal of Pharmaceutical Quality Assurance. 12 (2021) 57-60.
9. Tanpure RR, Kolhe MH, Shinde GS, Bhor RJ, Analytical Method Validation for Simultaneous Estimation of Domperidone and Omeprazole by RP-HPLC Method, International Journal of Drug Delivery Technology. 13 (2023) 692-697.
10. Prabhakar M, Vamshi RM, Shirisha RN, Neha B, Venkateswara RP, Akiful HM, Vasudha B, Narender B. Development and Validation of UV Spectrophotometric Method for the Determination of Bisoprolol in Bulk Material and in Tablets. International Journal of Biology, Pharmacy and Allied Sciences. 12(6) (2022) 2822-2834.