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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF EFAVIRENZ IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Two Simple, fast and reliable spectrophotometric methods were developed for determination of efavirenz in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in methanol. The quantitative determination of the drug was carried out using the area under curve (AUC) method at selected area between 243 to 253nm because the linearity was obtained within these areas with good reproducibility of results and second order derivative method measured at 239 nm. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of efavirenz using 5-40 µg.mL-1 (r^2 = 0.9995 and r^2 = 0.9993) for AUC method and second order derivative spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. These methods were successfully applied to pharmaceutical formulations because no interferences from tablet excipients were found. The proposed methods were found to be simple, sensitive, accurate, precise, rapid and economical for the routine quality control application of efavirenz in pharmaceutical formulations.

KEYWORDS

Efavirenz, Area under curve method, Second order derivative spectrophotometry and validation.

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INTRODUCTION

Efavirenz (EFZ) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) (Figure No.1). These drugs act by inhibiting HIV's reverse transcriptase enzyme, but in a different way from the nucleoside analogue drugs like AZT (zidovudine, Retrovi), abacavir (Ziagen), d4T (stavudinne, Zerif), 3TC (lamivudine, Epivir), ddc (Calcitabine, Hivid) and ddI (didanosine, Videx / VidexEC). Similar to

zidovudine, EFZ inhibits the activity of viral RNA – directed DNA polymerase. Antiviral activity of EFZ is dependent on intracellular conversion to the active triphosphorylated form. The rate of EFZ phosphorylation varies, depending on cell type. It is believed that inhibition of reverse transcriptase interferes with the generation of DNA copies of viral RNA, which, in turn, are necessary for synthesis of new virions. Intracellular enzymes subsequently eliminate the HIV particle that previously had been uncoated and left unprotected, during entry into the host cell. Thus, reverse transcriptase inhibitors are visustatic and do not eliminate HIV from the body. Even though human DNA polymerase is less susceptible to the pharmacologic effect of triphosphorylated Efavirenz, this action may nevertheless account for some of the drug's toxicity. Chemically efavirenz is known as 8-chloro –5-(2-cyclopropylethynyl)-5-(trifluoromethyl)-4-oxa-2-azabicyclo [4.4.0] deca- 7, 9, 11-trien-3-one.

Literature survey reveals a RP-HPLC method[1-2] for the determination of EFZ in combination with other drug and a method for Spectrophotometric estimation[3-5] and HPTLC[6-8] estimation of EFZ in its pure form and tablet dosage form. So far, there was no derivative spectrophotometric method reported for the estimation of efavirenz in pharmaceutical dosage form. We have developed zero and first order derivative spectroscopic method for estimation of EFZ in its pure and tablet dosage form[9]. In continuation with our work in this paper we deal with development and validation of an area under curve method and a second order derivative spectrophotometric method for the assay of EFZ from its bulk drug and in pharmaceutical dosage forms.

MATERIALS AND METHODS

UV-Visible double beam spectrophotometer, Jasco model 2201 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 nm matched quartz cell was used. The commercially available tablets, Sustiva (Label claim: Efavirenz-600mg) was procured from local Market.

Preparations of drug stock solution

Accurately about 10mg EFZ was weighed and transferred to 100 ml volumetric flask. To it 50ml of methanol was added to dissolve the drug completely with vigorous shaking. Then the volume was made up with the same solvent up to the mark to give the drug stock solution of concentration. 100 µg /ml.

Preparations of standard drug dilutions

From the stock solution of EFZ appropriate volumes giving concentrations which follow the Beer - Lambert's range were pipetted out and transferred to 10 ml volumetric flaks. The volume was made up to the mark with methanol to give the samples of desired concentrations.

Development of methods

Method I

Area under curve (AUC) method

By appropriate dilution of the standard stock solutions, working standard solutions of suitable concentrations were prepared accurately to determine the range for analysis (area under the curve). The standard solutions were then scanned in the spectrum mode of the instrument from 400nm to 200nm. The absorbance maxima of these solutions were found with a sharp peak at wavelength 248 nm (λ_{MAX} of EFZ in zero order spectrums). The area under the curve between 243 nm to 253nm was selected (figure 02) for the calculation because the linearity was obtained within this area with good reproducibility of results. The area between 243 nm to 253 nm was measured for each solution (Figure No.2).

Preparation of working curve

A series of volumetric flasks of 10ml capacity were arranged. To each of these flasks 0.5,1,2,3,4 ml of the drug stock solution were added. The volume was made up with methanol. The area of these solutions was measured between 243 nm to 253nm against reagent blank in spectrum mode as shown in figure. A linear graph of area Vs concentration was obtained, which shows that there was an increase in area with increasing concentration of the drug. The concentration range over, which the drugs obeyed Beer-Lambert's law was chosen as the analytical concentration range. Here, the concentration range

was found to be 5 to 40 µg/ml for EFZ. The working curve equation for EFZ was $y=0.5629_X$- 0.3823 r^2 = 0.9993. Using the standard working curve equation, the unknown concentrations of the drug were determined in bulk and formulations. The standard calibration table and curve for EFZ in AUC method is given in Table No.1, 2 and Figure No.3.

Method II

Second order derivative spectroscopic method

Selection of analytical wavelength

By appropriate dilution of the standard stock solutions working standard solutions of suitable concentrations were prepared accurately. The standard solutions were then scanned in the spectrum mode of the instrument from 400nm to 200nm. The second order derivative spectrum was obtained with wavelength difference (n=1). To obtain good results, the wavelength selected should be such that at this wavelength, the absorptivity should be as large as possible. The absorbance maxima of these solutions were found with a sharp peak at wavelength 262 nm (λ_{MAX} of EFZ in second order derivative spectrum) and zero crossing at 248 nm (λ_{MAX} of EFZ in zero order spectrum). The absorbance of the peak at 262 nm was measured for each solution as shown in Figure No.4.

Preparation of working curve

A series of volumetric flasks of 10ml capacity were arranged. To each of these 0.5, 1, 2, 3, 4 ml of the drug stock solution were added. The volume was made up with methanol. The absorbance of the peak in second order derivative spectrum with n=1 of these solutions were measured at 262 nm against reagent blank. A linear graph of absorbance of the peak Vs concentration was obtained passing through the origin, which shows that there was an increase in absorbance with increasing concentration of the drug. The concentration range over, which the drugs obeyed Beer-Lambert's law was chosen as the analytical concentration range (5 to 40 µg/ml) for EFZ. The standard calibration table and curve for EFZ in second order derivative spectrum with n=1 is given in Table No.3, 4 and Figure No.5.

Analysis of tablet formulation

Method I: Area under curve method

For estimation of EFZ in tablet formulations, five tablets of each brand were weighed and triturated to fine powder. Tablet powder equivalent to 10 mg of EFZ was weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol. It was kept for ultrasonification for 45 min. Finally the volume was made up to the mark with quantity sufficient methanol; this was then filtered through Whatmann filter paper to get tablet stock solution of concentration of 100 µg /ml. Then 2ml aliquots of 100 µg/ml tablet stock solution were suitably diluted with methanol to get the final dilutions of 20 µg/ml. Analysis of EFZ tablet formulations were done in above concentration by calculating area between 243 nm to 253 nm in spectrum mode against reagent blank. The procedure was repeated six times for each tablet formulations. The results of the analysis of the tablet formulation are given in Table.

Method II: Second derivative spectroscopic method

For estimation of EFZ in tablet formulations in this method, the same procedure was followed to make the stock solution of various dilutions for each brand of tablet formulations Analysis of EFZ in the tablet formulation was done in above concentration at 239nm against reagent blank in quantitation mode. The procedure was repeated six times for each tablet formulations.

Validation of developed method

Checking the AUC method

The method was checked by analyzing solutions containing known concentration of the drug. Standard sample solutions were prepared from the standard stock solution by pipetting out aliquots of 0.5,1,2,3,4 ml and transferred in 10ml volumetric flasks. The dilutions were made by using methanol to give the samples of concentrations 5, 10, 20, 30, 40 µg /ml. These samples were analyzed using standard calibration curve by measuring the area between 243 nm to 253 nm in AUC method using reagent blank. Good result was obtained (Table No.7) and hence the method was applied for the

analysis of EFZ in bulk and marketed tablet formulations (Table No.5).

Checking the Second order derivative method

The method was checked by analyzing solutions containing known concentration of the drug. Standard sample solutions were prepared same as that of previous method. These samples were analyzed against standard calibration curve using quantitation mode at 262 nm in second order derivative spectrum using reagent blank. Good results were obtained (Table No.8) and hence the method was applied for the analysis of EFZ in bulk and marketed tablet formulations (Table No.6).

Statistical validation and Recovery studies of developed method

Statistical validation

To check the degree of precision of the method, suitable statistical evaluation was carried out. Six samples of the each tablet formulation were analyzed as per the procedure given above. The standard deviation (S.D.), coefficient of variation (C.O.V.) and standard error (S.E.) were calculated.

Recovery studies

To check the accuracy of the proposed method, recovery studies were carried out at 80,100 and 120% of the test concentration as per ICH guidelines.

To perform recovery studies at 80% of the test concentration, a preanalayzed tablet sample containing 10 mg of EFZ was weighed. To it 8 mg of standard EFZ was added, the mixture was mixed thoroughly. From this pool, sample powder containing quantity equivalent to 10 mg of EFZ was weighed and transferred to a 100 ml volumetric flask. To it 50ml of methanol was added and the content was kept for ultrasonication to shake. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper. The sample mixture was then analyzed as per the procedure given for tablets. Similarly to perform recovery studies at 100% and 120% of the test concentration, a preanalyzed tablet sample containing 10mg of EFZ was weighed. To it 10 mg of standard EFZ (for 100% recovery) and 12 mg of standard EFZ (for 120% recovery) was added separately. The powders were mixed properly. From this pool, sample powder containing quantity equivalent to 10 mg of EFZ was weighed separately for 100% recovery and 120% recovery respectively. The powder then transferred to 100 ml volumetric flasks separately. The sample dilution and analysis was performed as per the procedure given under Tablet. The recovery study was performed three times at each level for the tablet formulations.

Area under the Curve (AUC) Method

The results of the analysis of tablet formulations by area under the curve (AUC) method are given below in Table No.7.

The % mean, standard deviation (S.D.), coefficient of variation (C.O.V.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The C.O.V. is also less than 2% as required by USP and ICH guidelines.

Recovery studies

The results of analysis of recovery studies and its statistical validation are given in Table No.9 and Table No.10 respectively.

The result of the recovery studies indicates high degree of accuracy of the proposed method.

Second Order Derivative Spectroscopy

The results of the analysis of tablet formulations by first order derivative spectroscopy method are given below in Table No.11.

Average of six readings

The % mean, standard deviation (S.D.), coefficient of variation (C.O.V.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The C.O.V. is also less than 2% as required by USP and ICH guidelines.

Recovery studies

The results of analysis of recovery studies and its statistical validation are given in Table No.13 and 14.

The result of the recovery studies indicates high degree of accuracy of the proposed method.

RESULTS AND DISSCUSSION

Linearity of EFZ is 5-40 µg/ml according to area under curve and second order spectrum. The coefficient of correlation for EFZ at calculated area

of 243 to 253nm in area under curve method is 0.9993 and coefficient of correlation of EFZ according to second order derivative method is 0.9995 at 248nm. EFZ shows good regression values at their respective wavelengths and area, also the results of recovery study revealed that any small change in drug concentration in the solution could be accurately determined by the proposed methods. Results are quoted in Table No.1-4.

Percentage estimation of EFZ in tablet dosage form by area under curve method is 99.88 and by second order derivative method is 99.90 with standard deviation ≤2 as shown in Table No.8 and 12.

The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods is in good agreement with their respective label claims, which suggest non interference of formulation additives in estimation as shown in Table No.9 and 13.

Precision is determined by studying the repeatability. Repeatability result indicates the precision under the same operating condition over a short interval of time and inter assay precision. The standard deviation, coefficient of variance and standard error is calculated for EFZ. The result is quoted in Table No.10 and 14.

Table No.1: Standard calibration table for EFZ in AUC method

S.No	Concentration (µg/ml)	AUC
1	5	2.6381
2	10	5.2326
3	20	10.554
4	30	16.4655
5	40	22.299

Table No.2: Optical characteristics of EFZ by AUC Method

S.No	Title	Mean	S.D.	C.O.V.	S.E.M.
1	R^2 Value	0.9990	0.0001835	0.01836	$0.0749x10^{-3}$
2	Y-intercept	0.3823	0.1991	52.0795	0.08127
3	Slope	0.5629	0.03227	5.7328	0.01317

Table No.3: Standard calibration table for EFZ

S.No	Concentration (µg/ml)	Absorbance
1	5	0.004
2	10	0.008
3	20	0.015
4	30	0.023
5	40	0.030

Table No.4: Optical characteristics of EFZ by second order derivative method

S.No	Title	Mean	S.D.	C.O.V.	S.E.M.
1	R^2 Value	0.9984	0.001118	0.1119	0.0004564
2	Y-intercept	0.00055	0.0001643	29.8727	$0.0670x10^{-3}$
3	Slope	0.00075	$0.0547x10^{-3}$	7.2933	$0.0223x10^{-3}$

Table No.5: Analysis of standard EFZ

S.No	Amount present (µg/ml)	Amount found (µg/ml)*	% of drug found*
1	5	4.967	99.34
2	10	10.019	100.19
3	20	20.016	100.08
4	30	30.0125	100.41
5	40	39.856	99.64

***Average of six readings**

Table No.6: Analysis of standard EFZ

S.No	Amount present (µg/ml)	Amount found (µg/ml)*	% of drug found *
1	5	5.0249	100.50
2	10	9.946	99.46
3	20	19.980	99.90
4	30	30.193	100.64
6	40	39.549	98.87

***Average of six readings**

Table No.7: Analysis of tablet formulation

S.No	Tablet Sample Name	Amount Present (mg /tab)	Amount Taken (mg)	Amount found (mg / tab)	Percentage of label claim (%)
1	Efavirenz	600	10	9.994	99.94
2		600	10	9.977	99.77
3		600	10	9.994	99.94
4		600	10	9.998	99.98
5		600	10	9.980	99.80
6		600	10	9.985	99.85

Table No.8: Statistical evaluation by AUC method

S.No	Tablet Sample Name	% Mean *	S.D.*	C.O.V.*	S.E.*
1	Efavirenz	99.88	0.08556	0.08566	0.03493

***Average of six readings**

Table No.9: Recovery Studies

S.No	Tablet Sample Name	Level of Recovery (%)	Amount present (mg/tab)	Amount Taken (mg)	Amount of Std Added (mg)	Total Amount recovered (mg)	% Recovery
1	Efavirenz	80	600	10	08	18.51	102.86
			600	10	08	17.99	99.96
			600	10	08	18.01	99.98
		100	600	10	10	20.02	100.10
			600	10	10	20.16	100.82
			600	10	10	19.50	97.48
		120	600	10	12	22.00	100.00
			600	10	12	21.99	99.99
			600	10	12	21.99	99.99

Table No.10: Statistical validation of recovery studies

S.No	Tablet Sample Name	Level of Recovery (%)	(%) Mean*	S.D.*	C.O.V.*	S.E.*
1	Efavirenz	80	100.93	1.669	1.6536	0.9634
2		100	99.47	1.758	1.7673	1.015
3		120	99.99	0.00577	0.00577	0.003333

Where *n=3 at each level of recovery.

Table No.11: Analysis of tablet formulation

S.No	Tablet Sample Name	Amount Present (mg /tab)	Amount found (mg / tab)	Percentage of label claim (%)
1	Efavirenz	600	583.59	97.27
2		600	604.23	100.71
3		600	603.75	100.62
4		600	604.92	100.82
5		600	604.65	100.77
6		600	595.20	99.20

Table No.12: Statistical evaluation by second order derivative spectrum method

S.No	Tablet Sample Name	% Mean *	S.D.*	C.O.V.*	S.E.*
1	Efavirenz	99.90	1.427	1.4284	0.5827

Table No.13: Recovery Studies

S.No	Tablet Sample Name	Level of Recovery (%)	Amount present (mg/tab)	Amount Taken (mg)	Amount of Std Added (mg)	Total Amount recovered (mg)	% Recovery
1	**Efavirenz**	80	600	10	08	18.18	101.04
			600	10	08	18.38	102.09
			600	10	08	18.18	101.01
		100	600	10	10	20.21	101.06
			600	10	10	20.42	102.10
			600	10	10	20.20	100.99
		120	600	10	12	22.23	101.05
			600	10	12	22.23	101.06
			600	10	12	22.10	100.45

Table No.14: Statistical validation of recovery studies

S.No	Tablet Sample Name	Level of Recovery (%)	(%) Mean*	S.D.*	C.O.V.*	S.E.*
1	**Efavirenz**	80	101.38	0.6151	0.3551	0.6067
2		100	101.38	0.6216	0.3589	0.6131
3		120	100.85	0.3493	0.2017	0.3463

Where *n=3 at each level of recovery.

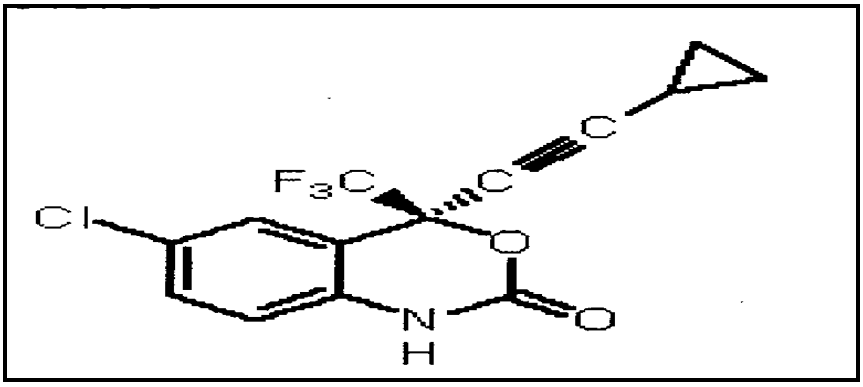

Figure No.1: Efavirenz

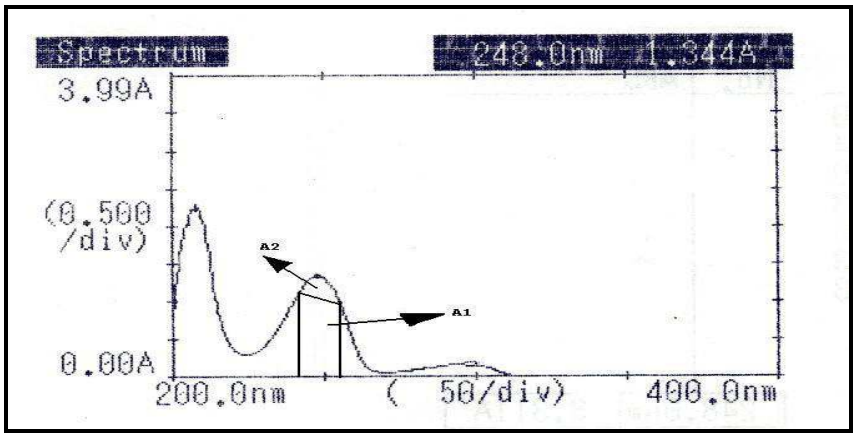

Figure No.2: Wavelength range selected for AUC method EFZ

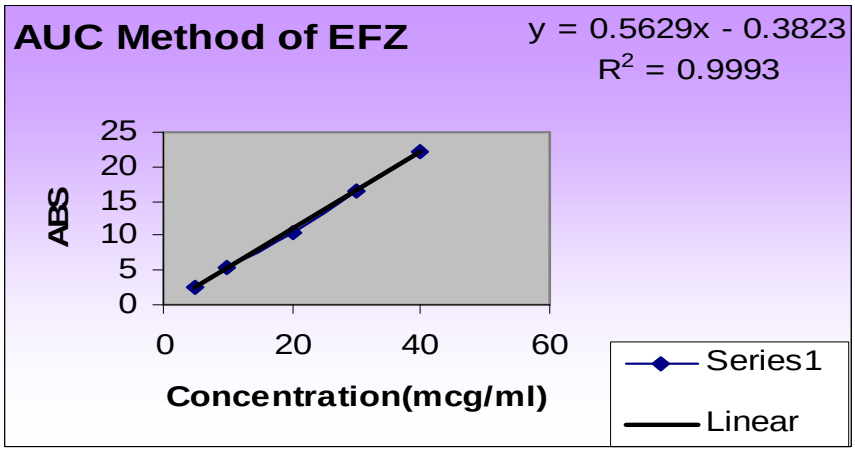

Figure No.3: Calibration curve of EFZ in AUC Method

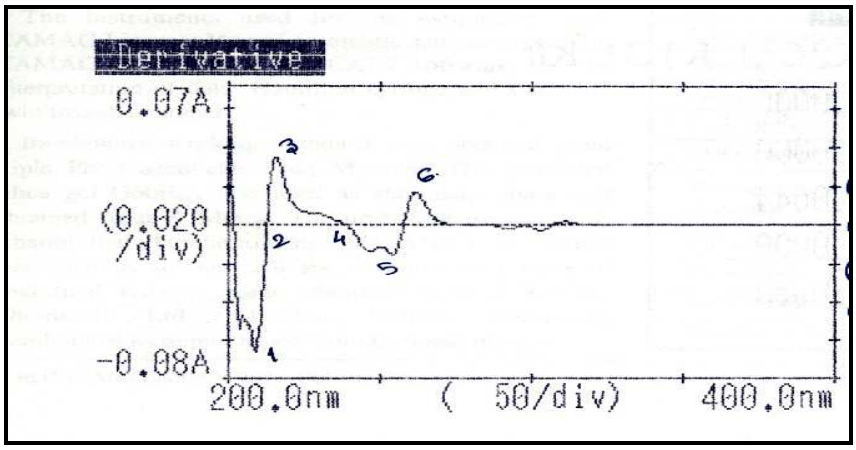

Figure No.4: Second order derivative spectrum of EFZ with n=1

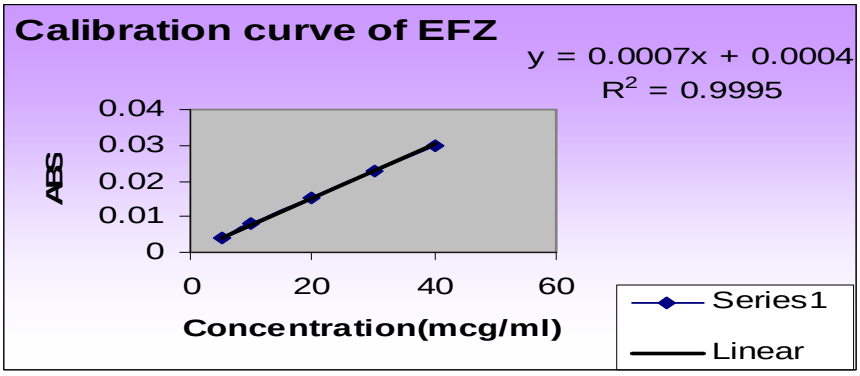

Figure No.5: Calibration curve of EFZ in second order derivative spectrum

CONCLUSION

The both developed methods are found to be fast, sensitive, precise, and reproducible for estimation of EFZ in bulk and pharmaceutical dosage forms. The methods are validated as per the ICH Guidelines. It is concluded that this method can be used by the industries and academic institutions for their combination drug estimation, which is rapid as well as novel.

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