STATISTICAL ASSURANCE OF PROCESS VALIDATION BY ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR EFAVIRENZ TABLETS AND BLEND

D.Kumaraswamy,*[1]Rajasridhar Rao[2],B.StephenRathinaraj[1],Ch.Rajveer[1],S.Sudharshini[1]

[1]Department of Pharmaceutical Chemistry, Vaagdevi College of Pharmacy, Warangal, A.P
[2]DepartmentofPharmaceutics, SR college of pharmacy, Warangal, A.P

ABSTRACT: A new simple, rapid and reliable UV Spectrophotometry method was developed and validated for the estimation of Efavirenz in blend & Tablets formulations. The method was based on simple UV estimation in cost effective manner for regular laboratory analysis. The instrument used was Perkin Elmer, UV Spectrophotometer (Lambda 25) and using 0.1 N NaoH as solvent system. Sample were analysed using UV Win Lab 5.2.0 Software and matched quartz cells 1 cm and was monitored at 302 nm. Linearity was obtained in the concentration range of 2 - 10 mg mL–1 for Efavirenz. The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of this method. Spectrophotometric interferences from the Tablets excipients were not found. The results of blend uniformity and content uniformity, done on process validation batches samples.

Key Words: UV Spectrophotometer, Efavirenz, Process Validation, Tablets Formulations, Quantitative analysis.

INTRODUCTION

Rizatriptan benzoate is chemically described as: N, N-dimethyl-5-(1H-1, 2, 4-triazol-1-ylmethyl)-1H-indole-3-ethanamine mono benzoate. It is a selective 5- hydroxyl triptamine -1B/1D receptor agonist[1] to relive migraine headaches. Its empirical formula is $C_{15}H_{19}N_5.C_7H_6O_2$ and its molecular weight is 391.47. Current theories on the etiology of headache suggest that symptoms are due to local cranial vasodilatation and/or to the release ofvasoactive and pro-inflammatory peptides from sensory nerve ending in an activated trigeminal system [2-4].The literature reveals that various methods for the determination of rizatriptan benzoate and pharmaceutical validations among these methods are UV,[5] LC-MS and LC-MS/MS [6], HPLC method for rizatriptan benzoate [7],Application and Development of Improved RP-LC-DAD for Rizatriptan and its degradation products [8], a method based on LC/MS/MS [9] using the UV detector were reported. The objective of this investigation is to develop, two simple, accurate and economical UV-spectrophotometric methods for estimation of Efavirenz using 0.1 N NaoH in which drug have good solubility. Process validation samples (blend and Tablets) are withdrawn at all stages and for all three validation batches for which analysis was performed using developed method.

EXPERIMENTAL:

Instrument:

For method, Perkin Elmer UV-Vis spectrophotometer (Lambda 25, spectral bandwidth 1nm) with 10 mm matched quartz cells; Shimadzu, Electronic Weighing Balance (AUX – 220), Oscar Ultrasonic Cleaner, Sonicator (Micro Clean 103) were used.
Reagent: Sodium Hydroxide (A.R.)

PROCEDURE

Method of analysis:

Standard stock solution of Efavirenz was prepared by dissolving 55 mg drug in 100 mL 0.1 N NaoH (i.e.550 µg/mL). Aliquot of these solutions were further diluted to obtain concentrations of 5.5 µg /mL for Efavirenz and scanned in the UV-range. From the spectra, wavelength 302 nm (λmax of Efavirenz) was selected. As reported in Figure1. The linearity was observed in the concentration range of 2- 10 µg/mL for Efavirenz. The absorptivity coefficient of drug at desired wavelengths was determined and the results are presented in Table1. The spectral data from this scan was used to determine the concentration of drug in blend and Tablets sample solutions.

Table: 1 Absorptivity A (1%, 1Cm) Values of Efavirenz at 302 nm

Concentration µg/ml	Absorbance	A(1%,1cm) Mean ± S.D	Molar Absorptivity (Mean ± S.D)
6	0.5874	979.11 ± 0.270	36234.93 ±11.87

*Mean of Six Concentrations.

Analyses of Process validation samples (Blend and Tablets formulation)

Twenty Tablets were weighed ,crushed in to powder and an amount of powder equivalent to 250 mg Efavirenz was transferred to a 100 mL calibrated volumetric flask, extracted with 0.1 N NaoH by shaking mechanically (for Content Uniformity). Similarly blend equivalent to 250 mg Efavirenz was transferred to a 100 mL calibrated volumetric flask, extracted with 0.1 N NaoH by shaking mechanically (for Blend Uniformity). The solution was diluted to mark with the same solvent and filtered through Whatmann filter paper. (no. 41). Aliquot portion of this solution was diluted to get concentration of 6 µg/mL of Efavirenz. Absorbance of the sample solutions were recorded, at 302 nm respectively (Perkin Elmer, Lambda 25). And, the concentrations of drug in samples were determined, by using calibration curve. The concentration of each drug was determined by analysis of spectral data of the sample solutions with reference standards. The results are reported in Table 2.

Table: 2 Results of Assay

Result Of Assay Label Claim (mg/Tab)	% Label Claim*	± SD	%RSD	SE
250mg	100.74	0.88	0.83	0.14

* Mean of Five Estimation.

Recovery Studies

The recovery studies were carried out at three different level i.e. 80,100 and 120%. It was performed by adding known amount of standard drug solutions of Efavirenz to preanalysed Tablets solutions. The resulting solutions were then reanalyzed by proposed methods. The results of recovery studies are shown in Table 3.

Table: 3 Results of Recovery studies

S.No	Amount Of Drug Added (µg/mL)	% Recovery* ± SD	%RSD
1	3.2	99.58± 0.41	0.41
2	4.0	99.1± 0.60	0.64
3.	4.8	98.8 ± 0.88	0.89

RESULTS AND DISCUSSION:

The proposed methods are simple, sensitive, accurate, precise, reproducible, economic and rapid for simultaneous analysis of Efavirenz in Tablets. Accuracy of the method was evaluated by carrying out recovery studies. Low values of %RSD are indicative of high precision of the methods. The repeatability and ruggedness study signifies the reproducibility of the method as shown in Table 4.

Based on the validation study data, it can be concluded that the proposed method is accurate and precise for the analysis of drug. No interference was found from excipients used in Tablets formulation and hence the method is suitable for analysis of blend and Tablets formulation. Process validation samples, blend uniformity was found to be good within and between all three validation batches as shown in Table 5. Formation of Tablets, sample for content uniformity were collected at three stages (initial, mid, end) for all three validation batches, results for which show that there is uniformity in dosage units within batch and similarity between batches as shown in Table 6.

Table: 4.Results of Repeatability and Ruggedness studies.

Parameters	% Recovery*± RSD
Precision %RSD	
Intra-Day (n=3)	0.40-1.46
Inter-day (n=3)	0.72-1.39
Repeatability (n=6) % RSD	0.89
Ruggedness (n=5)	
Analyst I	0.65
Analyst II	0.74

Table: 5.Blend Uniformity * (% Assay for Each Sample)

	Batch I	Batch 2	Batch 3
Mean	100	98.4	102.1
Min.	98.6	98.1	101.5
Max	0.77	0.3	0.53

*Final blend analysed for 6 locations from Rapid Mixing Granulator.

Table: 6.Content Uniformity * (% Assay for Each Sample)

S.No	Batch 1 Stage			Batch 2 Stage			Batch 3 Stage		
	1	2	3	1	2	3	1	2	3
Mean	99.9	100.2	100.1	99	100.3	101.3	102.7	103	103.2
Min	99.9	99.5	99.6	97.4	98.4	100.2	102	102.6	102.7
Max.	98.9	100.6	100.6	100.2	102.3	103.8	104.1	103.9	102.6
%RSD	0.50	0.4	0.3	1.1	1.2	0.7	0.7	0.5	0.4

* Ten Units individual assay was analyzed for each stage of all the batches.

ACKNOWLEDGEMENTS

The authors are thankful to Ranbaxy Laboratories Ltd.(Dewas, India) for providing gift sample of drug, Process Validation samples of blend and Tablets for this work and College of Pharmacy and Technology Management for providing necessary facilities.

REFERENCES

1.Goldberg MR, Lee Y, Ermlich S.(2000), J Clin Pharmacol; 40: 74-83.
2. Bou J, Domenech T, Puig J, Heredia A, Gras J, Fernandez-Forner D, Beleta J. Palacios (2000) JM. Eur J Pharmacol 410**:** 33-41.
3. Emile H, Joseph H, Hashmonai D.(2003), J Emer Med 3: 245-249.
4. Sharma A, Jusko WJ, Fulmor IE(1999). J Clin Pharmacol 39: 685-694.
5. Ter Heine, Rob; Alderden-Los, Carolien G.; Rosing, Hilde;Hillebrand, Michel J. X.; van Gorp, Eric C. M.; Huitema,Alwin D. R.; Beijnen, Jos H. (2007), Rapid Communications in MassSpectrometry, 21(15), 2505-2514.
6. Chen Y, Miao H, Lin M, Fan G, Hong Z.(2006), J Chromatogr; 844: 268-277.
7. Joseph SR, Bharathi CH, Joseph P, Naveen Kumar P, Sharma HK, Kalpesh. (2009) ,J Pharm Biomed; 1: 1156-162.
8. Zecevic M, Jocic B, Zivanovic L, Protic A. (2008), J Chromatogr; 0009-5893: 911-918.
9. Vyas KP, Halpin RA, Geer LA. (2000),J Drug Met Disp; 28: 89-95.