Research Article

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VALIDATED ZERO AND FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF POORLY SOLUBLE ANTIRETROVIRALS

Himaja Malipeddi*, Kalpana Jayapalu, Chaitra Muralidhara
Pharmaceutical Chemistry Division, SAS, VIT University, Vellore, Tamilnadu, India

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***Corresponding author**
Dr (Mrs). M. Himaja, Professor, Pharmaceutical Chemistry Division, SAS, VIT University, Vellore-632014. Tamil Nadu, India
Email:drmhimaja@gmail.com

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ABSTRACT

Two highly simple, sensitive and rapid derivative spectrophotometric methods were developed for the estimation of efavirenz and nevirapine in bulk and pharmaceutical dosage forms. The standard and sample solutions were prepared using methanol and distilled water in the ratio of 40:60 (azeotropic mixture). The spectrophotometric estimation of efavirenz and nevirapine was carried out using the using the zero order derivative values measured at 247 and 282 nm respectively and the first order derivative values measured at 257 and 299 nm respectively. Calibration graphs constructed at their wavelengths of determination were linear within the concentration range of 4-24 µg/ml for zero order and first order derivative spectrophotometric method. Both the proposed methods have been extensively validated as per ICH guidelines. No significant difference between the performance of the proposed methods regarding the mean values and standard deviations were noticed and is suitable for the routine quality control application of active drugs in pharmaceutical formulations.

Keywords: Efavirenz, Nevirapine, Zero order derivative spectrum, first order derivative spectrum, validation.

INTRODUCTION

Efavirenz is chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3, 1-benzoxazin-2-one. It is practically insoluble in water. Efavirenz is an antiretroviral drug which belongs to a class of non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus (HIV) type-1. The drug is used in combination with other anti-retroviral agents for the treatment of HIV-1 infection in children and adults[1-2]. Nevirapine (NVP) is 11-cyclopropyl-4-methyl-5,11-dihydro-6Hdipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one. Nevirapine belongs to a class of drugs known as Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) with activity against HIV-I[3]. It is practically insoluble in water. Literature study reveals that very few analytical methods have been reported for the estimation of efavirenz and nevirapine in bulk or in pharmaceutical formulations[4-11]. Both the drugs selected for the study are practically insoluble in water. In the existing methods, the organic solvents used for the estimation were costly and not environment-friendly. So we applied concept of azeotropic mixture and combined an organic solvent (methanol) with water and developed simple and cost effective spectrophotometric methods[12] for the estimation of nevirapine and efavirenz. These methods were successfully applied to pharmaceutical formulations and 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 the active drugs in pharmaceutical formulations.

MATERIALS AND METHODS

Efavirenz and nevirapine standard were procured as gift samples from Strides Arcolab Ltd. Bangalore, Karnataka, India and was used without further purification. All chemicals and reagents used were of analytical grade. All stock solutions were prepared using double distilled water. Spectrophotometric measurements were performed using a Jasco V 670 UV/VIS/NIR diode array spectrophotometer (scan speed 400 nm/min and wavelength interval 1 nm), associated with Spectra manager software (Jasco, Japan).

Method Development

Preparation of Standard and Sample Solutions

Stock solution of 800 µg/ml of efavirenz and nevirapine was prepared in azeotropic mixture (Methanol: Water, 40:60 v/v), for zero order and first order derivative spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with azeotropic mixture in a concentration range of 4, 8, 12, 16, 20 and 24µg/ml for zero order and first order derivative spectrophotometric measurements. Methanol: Water, 40:60 v/v was used as a blank solution.

Assay Procedure

A total of 20 tablets of efavirenz were accurately weighed and powdered. Powder equivalent to 10 mg was

accurately weighed and transferred to volumetric flask of 25 ml capacity. 15 ml of the mixture of methanol and water (40:60, v/v) was transferred to volumetric flask and sonicated for 5 min. The flask was shaken and volume was made up to the mark with the mixture of methanol and water (40:60, v/v). The above solution was filtered through Whatman filter paper (0.45 mm). From this solution, 5 ml was transferred to volumetric flask of 25 ml capacity. The volume was made up to the mark to get a concentration 80 µg/ml (Solution A). From the solution A, 1.5 ml was transferred to volumetric flask of 10 ml capacity. The volume was made up to the mark with the mixture of methanol and water (40:60, v/v) to give a solution containing 12 µg/ml for both zero order and first order derivative spectrophotometric methods. Same procedure has been used for the nevirapine assay.

Method A: Zero Order Spectroscopic Method

The solutions of efavirenz and nevirapine were scanned in the range from 200- 400 nm, the maximum absorbance was observed at 247 and 282 nm respectively and were applied for further drug analysis. The active drugs followed the Beer's- Lamberts law in the concentration range of 4-24 µg/ml.

Method B: First Order Derivative Spectroscopic Method

The standard drug solutions in the concentration range of 4-24 µg/ml was scanned in the first order derivative spectra. The first order derivative spectra showed maxima at 257 and 299 nm for efavirenz and nevirapine respectively. The amplitude of absorbance was measured and was plotted against concentration to give calibration curve, and regression equation was calculated. The amplitude was linear in the concentration range of 4-24 µg/ml.

Table 1: Intra and Inter day precision results

Parameters	Intraday precision				Inter day precision			
	Efavirenz		Nevirapine		Efavirenz		Nevirapine	
	S.D	%RSD	S.D	%RSD	S.D	%RSD	S.D	%RSD
Zero derivative	0.051316	0.22	0.00197	0.68	0.040415	0.29	0.0079	2.72
First derivative	0.001193	0.37	0.0001	0.94	0.001808	0.41	0.000208	1.93

Table 2: Accuracy results

Zero order derivative method									
Accuracy Level	Absorbance		Amount added(mg)	Amount found(mg)		% recovery		Mean recovery	
	Efavirenz	Nevirapine		Efavirenz	Nevirapine	Efavirenz	Nevirapine	Efavirenz	Nevirapine
80%	1.2746	0.2904	8	7.96	8.12	98.66	101.5	99.09	100.46
100%	1.3118	0.2936	10	9.92	9.93	99.55	99.3		
120%	1.3677	0.2890	12	11.98	12.08	99.08	100.6		
First order derivative method									
80%	0.0858	0.0103	8	7.97	7.92	99.6	99.0	99.62	99.82
100%	0.1258	0.0107	10	9.97	10.14	99.7	101.4		
120%	0.1901	0.0106	12	11.95	11.89	99.58	99.08		

Table 3: Assay results of active drugs in pharmaceutical formulation

Efavirenz			
Parameters	Amount of Tablet label claim	Drug content %	%RSD
Zero order	60mg	99.80	0.197
First order	60mg	99.10	0.915
Nevirapine			
Zero order	200 mg	99.55	0.6
First order	200 mg	99.46	0.5

Table 4: Regression analysis data and summary of validation parameters for the proposed methods

Parameter	Zero order		First order	
	Efavirenz	Nevirapine	Efavirenz	Nevirapine
Absorption maxima	247	282	257	299
Beer's-Lamberts range (µg/ml)	4-24	4-24	4-24	4-24
Regression equation y=mx+c	y=0.0475x-0.0297	y=0.0264x-0.0198	y=0.0253x+ 0.0021	y=0.0008x-0.0006
Slope(m)	0.0475	0.0264	0.0253	0.0008
Intercept(c)	0.0297	-0.0198	0.0021	-0.0006
Correlation coefficient (r2)	0.9994	0.9905	0.9990	0.9939
Mean Recovery %	99.09	100.46	99.62	99.82
Precision (% RSD)	0.22	0.68	0.37	0.94
Intermediate precision	0.29	2.72	0.41	1.93
LOD (µg/ml)	0.1132	0.5238	0.2011	0.5214
LOQ (µg/ml)	0.3442	1.5875	0.2989	1.5800

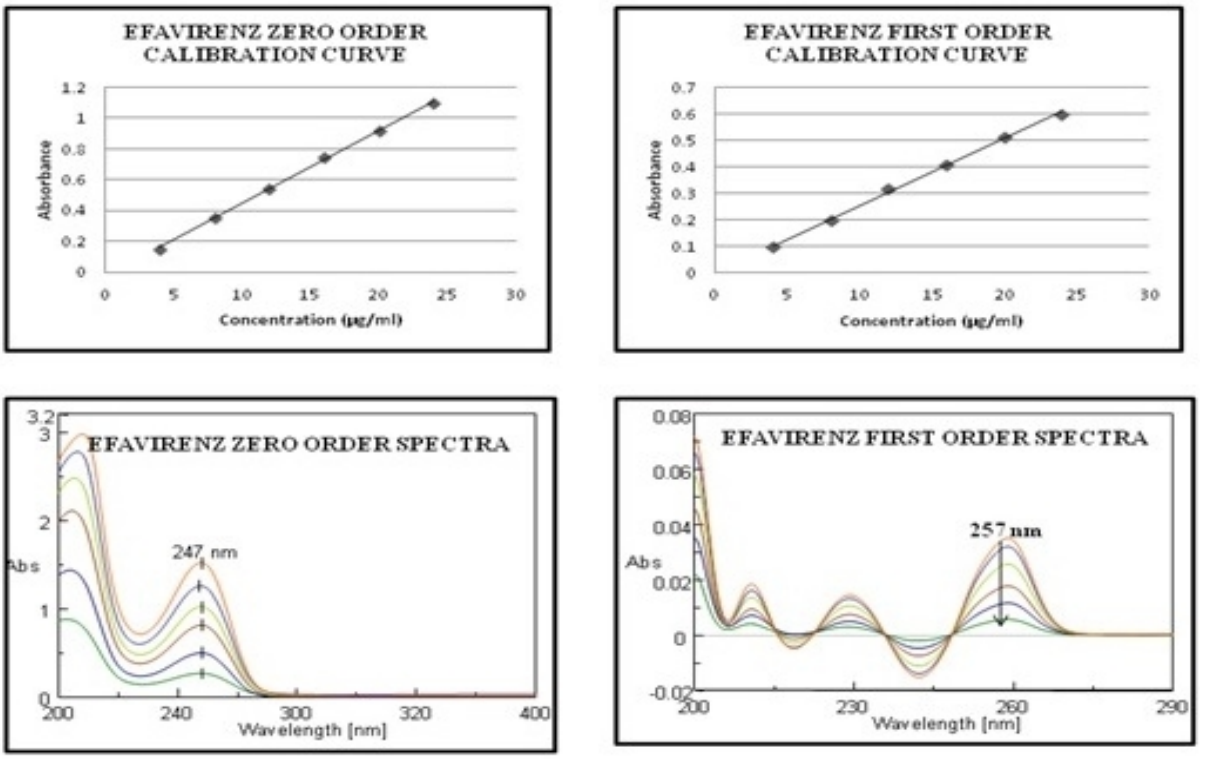

Figure 1: Efavirenz Calibration Curves

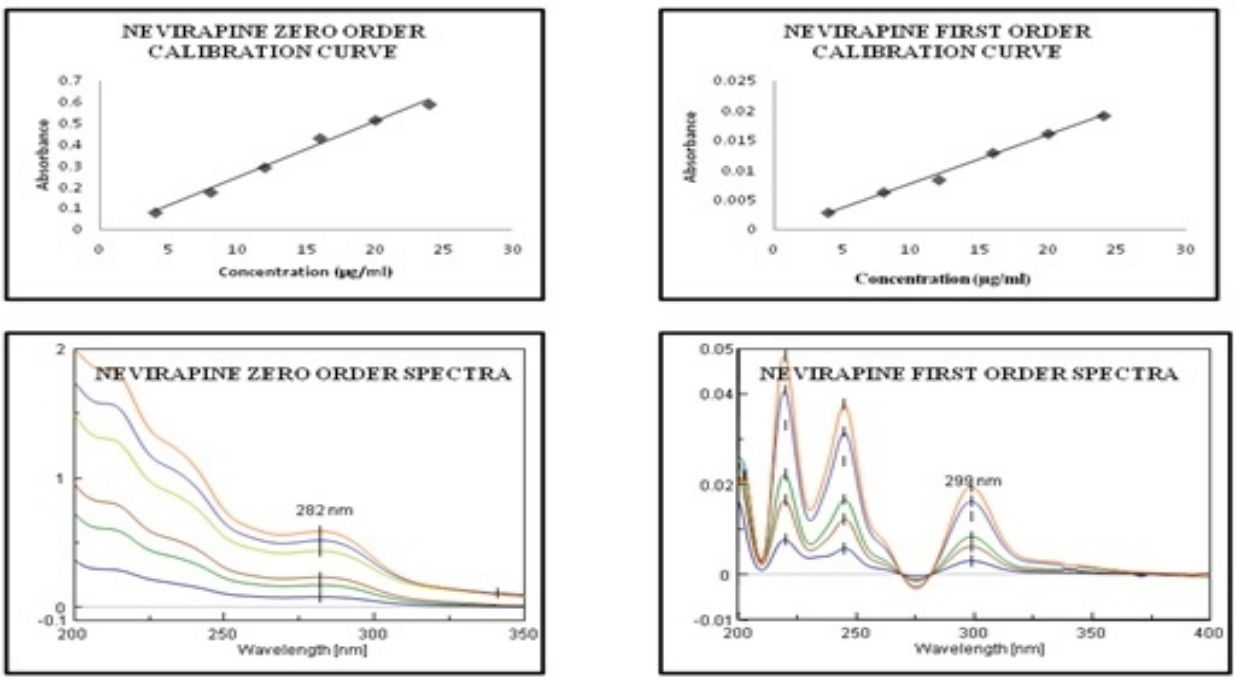

Figure 2: Nevirapine Calibration Curves

RESULTS AND DISCUSSION

The developed method was validated for linearity, range, precision, accuracy, specificity, LOD and LOQ according to the International Conference on Harmonization (ICH) guidelines[13]. The zero order and first order derivative spectra were recorded at the wavelength of 247 and 257 for efavirenz, 282 and 299 nm for nevirapine respectively.

Linearity and Range

The calibration curves were obtained by plotting the absorbance against the drug concentration and were subjected to least square linear regression analysis to obtain the calibration equations and correlation coefficients. From stock solutions 4-24 µg/ml concentration range solutions were prepared in azeotropic mixture**.** Under the experimental conditions described, the graph obtained for zero order and first order derivative spectra showed linear relationship for both the drugs. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves of efavirenz and nevirapine were y = 0.0475x - 0.0297 (r^2 = 0.9994) at 247 nm and y = 0.0264x - 0.0198 (r^2 = 0.9905) at 282 nm respectively for zero order derivative spectrophotometry and y = 0.0253x + 0.0021 (r^2 =0.999) at 257 nm and y = 0.0008x - 0.0006 (r^2 = 0.9939) at 299 nm respectively for first order derivative spectrophotometry. The range was found to be 4-24 µg/ml for both zero order and first order derivative spectrophotometric methods. The calibration curves are showed in figures 1 and 2.

Precision

To determine the precision of the method, standard drug solutions at a concentration of 12 µg /ml were analyzed each five times for both zero order and first order derivative spectrophotometric methods. Solutions for the standard curves were prepared freshly. Measured the absorbance and calculated the %RSD. The %RSD for the five replicates absorbance was found to be within the specified limits. Solutions for the standards were freshly prepared at the time of analysis. The % RSD for the area of five standard injections results should not be more than 2%. The precision results are tabulated in table 1.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3 σ / S and LOQ = 10 σ /S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ results of efavirenz and nevirapine for zero order and first order derivative methods are tabulated in table 4.

Recovery

Standard addition method was applied to study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage. This study was performed by addition of known amounts of active drug to reanalyzed solutions of commercial tablets. Measured the absorbance of the standard solution, accuracy 80%, accuracy 100% and accuracy 120% solutions and calculated the individual recovery and mean recovery values. The results are shown in table 2.

Analysis of the Marketed Formulation

No interference from the excipients commonly present in the tablets was observed during the analysis. The drug content of efavirenz was found to be 99.8% with a % R.S.D. of 0.197 and 99.1% with a % R.S.D. of 0.915 for zero order and first order derivative spectrophotometric methods respectively and the drug content of nevirapine was found to be 99.55% with a % R.S.D. of 0.6 and 99.46% with a % R.S.D. of 0.5 for zero order and first order derivative spectrophotometric methods respectively. It may therefore be inferred that degradation of efavirenz and nevirapine had not occurred in the marketed formulations that were analyzed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of the active drugs in pharmaceutical dosage form. The results are shown in table 3. The summary of the validation parameters is depicted in table 4.

CONCLUSION

Two spectrophotometric methods for quantifying efavirenz and nevirapine in bulk and formulation have been developed and validated as per ICH guidelines. The developed methods are selective, economically cheap and linear over the concentration range from 2µg/ml to 24µg/ml in the solvent of methanol and water (60:40). The developed methods can be concluded as accurate, sensitive and precise and can be easily applied for the routine quantification in pharmaceutical formulations.

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