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A new RP-HPLC method development and validation for simultaneous estimation of zidovudine and efavirenz in a pharmaceutical dosage forms

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ABSTRACT

The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for simultaneous estimation for Zidovudine and Efavirenz in pharmaceutical dosage forms. Chromatographic separation was done using symmetry C_{18} column having dimension of 150×4.6mm, having particle size of 5 µm, with mobile phase consisting of Phosphate buffer (KH_2PO_4 and K_2HPO_4) pH 3.5. pH adjusted to 3.5 with orthophosphoric acid and methanol (70:30 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 260nm. The retention times (RT) of Zidovudine and Efavirenz was found to be 2.463 and 3.762mins. The proposed method has been validated for accuracy, precision, linearity, robustness and range was within the acceptable limit according to ICH guidelines. Linearity for Zidovudine and Efavirenz was found in range of 20ppm-100ppm.The correlation coefficient was found to be 0.999 and 0.999(r^2≤0.999%) ,intermediate precision was found to be 1.3 for Zidovudine and 0.4 for Efavirenz. The repeatability studies were recover as 0.3% for Zidovudine and Efavirenz, then %recoveries for Zidovudine 98.4% and Efavirenz 99.0%, The method was found to be robust even by change in the mobile phase ±10% and in less flow condition. The developed method can be successfully employed for the routine analysis of Zidovudine and Efavirenz in pharmaceutical dosage forms.

Key words: Zidovudine, Efavirenz, RP-HPLC, Method development, Validation.

INTRODUCTION

Zidovudine is a 1-[(2R,4S,5S)-4-Azido-5-(hydroxymethyl)oxolan-2-yl]-5- methylpyrimidine-2,4-dione. It is an antiretroviral drug. Mainly used for human immunovirus (HIV) infections. Zidovudine is nucleoside reverse transcriptase inhibitor (NRTI) with activity against human immunodeficiency virus type 1(HIV-1). Zidovudine is phosphorylated to active metabolites that compete incorporation to viral DNA.

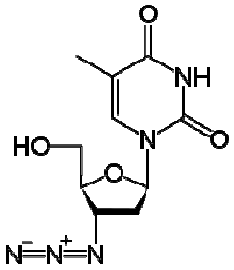

Figure 1: Structure of zidovudine

Efavirenz is a(4s)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)2,4- dihydro-1H-3-1-benzoxazin-2-one. Efavirenz is a oral nucleoside reverse transcriptase inhibitor NRTI) it is a synthetic purine derivative. Efavirenz was

originally approved for the HIV infections efavirenz inhibits the activity of viral RNA-directed DNA polymerase antiviral activity of efavirenz dependent on intercellular conversion to active triphosphorylated form. The rate of efavirenz phosphorylation varies, depending on cell type. Inter cellular enzymes subsequently eliminate HIV particle that previously have been uncoated and left unprotected during entry into the host cell.

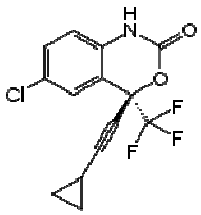

Figure 2: Structure of Efavirenz

MATERIALS AND METHODS

Apparatus

The liquid chromatographic system of waters (India) containing quaternary gradient pump, variable wavelength programmable PDA detector, and auto sampler with 10 μL fixed loop was used. For analysis a symmetryC_{18} column with 150 ×4.6 mm i.d. and 5 μm particle size was used as stationary phase

Reagents and Materials

Pharmaceutical grade Z idovudine and E favirenz were pursued as a gift sample from Limited, India. All chemicals and solvents of HPLC grade and were purchased from SD- Fine Chemicals. Hyderabad,

Preparation of Mobile Phase and Stock Solution

Mobile phase was prepared by accurately weighing 7.0gms of potassium Di hydrogen orthophosphate in to a 100ml beaker dissolved & diluted to 100ml with HPLC water. Adjust P_h 3.5 with orthophoshoric acid. Mix a mixture of above 300ml (30%) and 700 ml of methanol (70%) & degas with ultrasonic water bath for 5 min. Filter through 0.45 µ filter under vacuum filtration.

Stock solutions were prepared by accurately weighing 10 mg of ZDV and 10 mg of EFV and transferring to two separate 10 mL volumetric flasks containing 7 mL of mobile phase. The flasks were sonicated for 10 minutes to dissolve the solids. Further pipette out 0.6ml from the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with diluent Volumes were made up to the mark with mobile phase, which gave 100 µg/mL and 100 µg/mL of the ZDV and EFV respectively. A figure 3 and 4 represent the typical chromatogram of standard Zidovudine and E favirenz respectively.

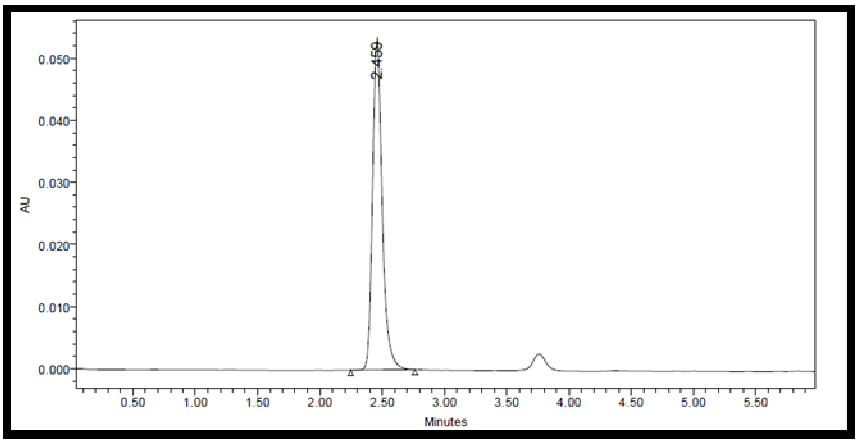

Figure 3: HPLC chromatogram of standard zidovudine

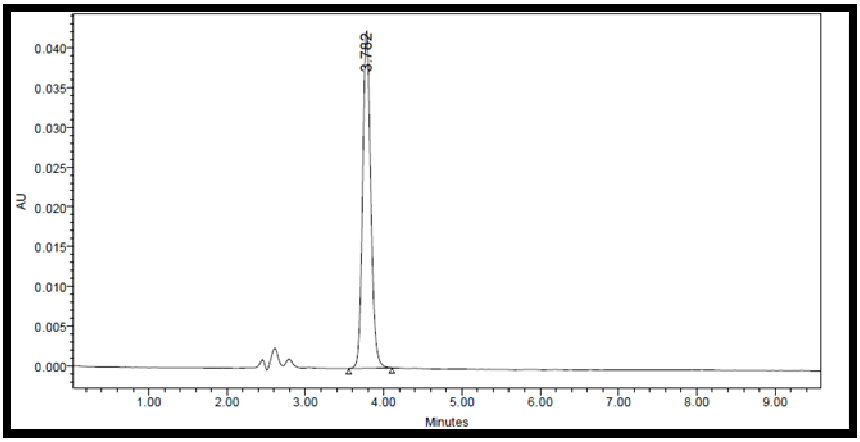

Figure 4: HPLC chromatogram of standard Efavirenz

Chromatographic Conditions

The symmetry C18 column (150 × 4.6 mm) equilibrated with mobile phase Methanol: phosphate buffer: (KH_2PO_4 and K_2HPO_4) (70:30 v/v), pH 3.5 adjusted with orthophosphoric acid was used. The flow rate was maintained at 1.0 mL/min, eluents were monitored with PDA detector at 260 nm, and the monitored injection volume was 20µ L. Total run time was kept for 6 min.

Method Validation

The method was validated for accuracy, precision, sensitivity, recovery, linearity and robustness. The method validation was performed as per ICH guidelines.

Linearity

Appropriate aliquots of the standard stock solutions of ZDV and EFV were pipette out and transferred to a series of 10 mL volumetric flasks respectively. The volume was made up to the mark with mobile phase to obtain working standard solutions of ZDV and EFV of concentrations 20ppm to 100ppm. The calibration curves were found to be linear and in adherence to Beer's law over the concentration range of 20-100 µg/mL for ZDV and 10-100 µg/mL for EFV. The solutions were injected using a 20 µL fixed loop system,and chromatograms were recorded. Calibration curves were constructed by plotting peak area Vs concentrations of the drug and regression equations were computed for ZDV and EFV.

The standard calibration tables and graphs for ZDV and EFV are shown in Table No. 1 and 2, Figure No. 5 and 6 respectively.

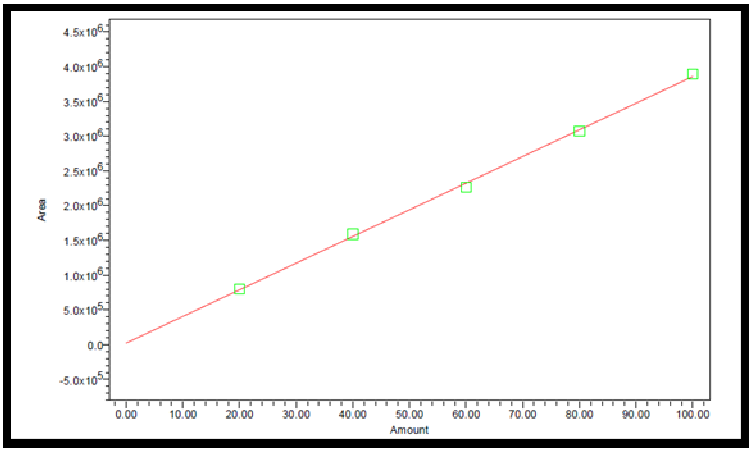

Figure 5: Calibration curve for ZDV

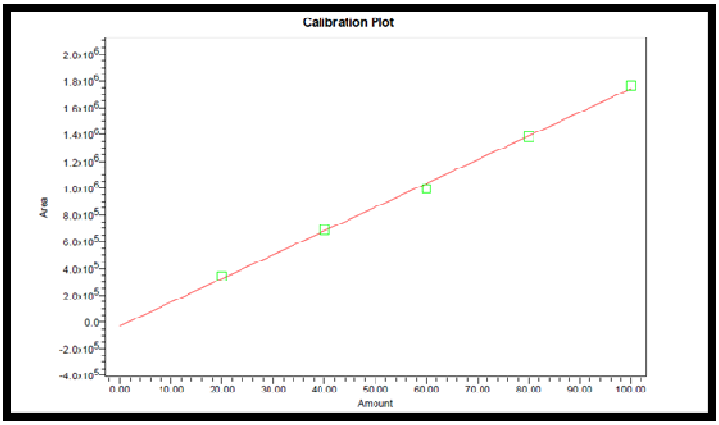

Figure 6: Calibration curve for EFV

Table 1: Calibration table for ZDV

S.NO	CONCENTRATION	AREA
1.	20	800199
2.	40	1589391
3.	60	2264300
4.	80	3071625
5.	100	3894075
Y-Intercept		77325
Correlation coefficient		0.999

Table 2: Calibration table for EFV

S.NO	CONCENTRATION	AREA
1.	20	800199
2.	40	1589391
3.	60	2264300
4.	80	3071625
5.	100	3894075
Y-Intercept		34682
Correlation coefficient		0.999

Precision

The repeatability studies were carried out by estimating response of ZDV (10 mg/mL) and EFV (10 mg/mL) five times and results were reported in terms of relative standard deviation. The intraday and interday precision studies (intermediate precision) were carried out by estimating the corresponding responses injecting 6 times on the same day and Different concentrations and the results were reported in terms of relative standard deviation

Accuracy

Accuracy was performed by recovery studies. The recovery studies were carried out at three concentration level 50%, 100%, 150% by standard addition method. The percentage recovery and standard deviation were calculated and reported in table No. 3.

Sensitivity

The sensitivity of measurement for ZDV and EFV was estimated in terms of the limit of quantitation (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOQ and LOD were calculated by use of the equations

.

$$LOD = 3.3 \text{ X } \frac{\sigma}{S}$$

$$LOQ = 10 \text{ X } \frac{\sigma}{S}$$

Where σ is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration curve.

Robustness
Robustness of the method was studied by deliberately changing the experimental conditions like flow rate temperature and percentage of mobile phase ratio. The study was carried out by changing ±10% of the mobile phase ratio and 0.18mL/min to 1.2ml/min of flow rate.

System Suitability
A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of ZDV and EFV to be performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a check standard were made. Area, retention time (Rt), tailing factor, asymmetry factor, and theoretical plates for the five suitability injections were determined.

RESULTS AND DISCUSSION

Optimization of Mobile Phase
Optimization of mobile phase was performed based on resolution of the drugs and, asymmetric factor, and theoretical plates obtained for ZDV and EFV. The mobile phase consisting methanol: phosphate buffer in order to find the optimum conditions for the separation of ZDV and EFV. After several trials, mobile phase of methanol phosphate buffer: (KH_2PO_4 and K_2HPO_4) (70:30 v/v), pH 3.5 adjusted with orthophosphoric acid was used selected which gave sharp, well-resolved peaks for ZDV and EFV (Figure 3,4).

The retention times (RT) of zidovudine and efavirenz was found to be 2.463 and 3.762mins respectively. The theoretical plates were found to be 5404.6, 7344.3 and tailing factor 1.2 and 1.1 mins respectively.

Method Validation
The calibration curve for ZDV was found to be linear in the range of 20-100µ g/mL with a correlation coefficient of 0.999. The calibration curve for EFV was found to be linear in the range of 20-100µ g/mL with a correlation coefficient of 0.999. Instrument precision was determined by performing injection repeatability test and the RSD values for ZDV and EFV were found to be 0.3% and 0.3%, respectively. The intermediate precision studies were carried out and the results are reported in Table 3. The low RSD value indicates that the method is precise.

The accuracy of the method was determined by calculating recoveries of ZDV and EFV by method of standard addition. The recoveries were found to be 101.2-101.8% and 101.4-101.4 % for ZDV and EFV, respectively. The results are reported in Table 3.

Table 3: Summary of validation parameters

Parameters	ZDV	EFV
Accuracy	99.6%	99.6%
Precision(%RSD)	0.3%	0.3%
Intermediate precision(%RSD)	1.3%	0.4%
Linearity	0.999%	0.999%
LOD(µg/ml)	2.93	3.02
LOQ(µg/ml)	9.91	10.1

The high values indicate that the method is accurate. The detection limits for ZDV and EFV were found to be 2.93 and 3.02 respectively while quantitation limits were found to be 9.91 and 10.1respectively. Robustness study was performed by deliberately changing the experimental conditions like flow rate from 0.8mL/min to 1.0 mL/min and 1.2 mL/min. The composition of mobile phase was changed by varying the proportion of methanol by ±10%, pH and temperature was changed. System suitability parameters such as the number of theoretical plates, resolution, and tailing factor were determined. System suitability test was carried out and the results are summarized in Table 4.

Table 4: System suitability test parameters for the proposed method

System suitability parameters	ZDV	EFV
Retension time	**2.463**	**3.762**
Tailing factor	**1.2**	**1.1**
Theoritical plate	**5404.6**	**7344.3**
Resolution		**8.3**

CONCLUSION

The objective of the work was to develop the simple, accurate, precise and sensitive HPLC method for the estimation of Zidovudine and Efavirenz in pharmaceutical dosage forms. From the results obtained by all parameters, it is concluded that developed RP-HPLC method is suitable for the simultaneous estimation of Zidovudine and Efavirenz in pharmaceutical dosage forms. The concentration of ZDV and EFV in pharmaceutical dosage forms could be satisfactorily determined using gradient RP-HPLC system with PDA detector. This method has been found suitable for the routine analysis of pharmaceutical dosage forms.

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