Original Article

Development and validation of UV Spectrophotometric method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical dosage form

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ABSTRACT

A simple, specific, accurate and precise UV spectrophotometric method has been developed for simultaneous estimation of lamivudine and efavirenz in pharmaceutical dosage form. The absorption maxima of the drugs were found to be at 271 and 247 nm for lamivudine and efavirenz respectively. Lamivudine and efavirenz obeyed Beer's law in the concentration range of 10-100 µg/ml and 10-70 µg/ml respectively. Different analytical parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined as per ICH guidelines. Limit of detection and quantification values for lamivudine 10 and 3.5 µg/ml and for efavirenz 10 and 3.0 µg/ml respectively. The recovery values between prescribed limit of 98-102% shows that method is free from interference of excipients present in formulation. The developed method was free from interferences due to excipients present in formulation and it can be used for routine quality control analysis.

Keywords: Lamivudine; efavirenz; UV spectrophotometric method; simultaneous equation method.

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs, which were introduced as antiretroviral agents for the treatment of infection with human immune deficiency virus (HIV). Additional drug classes were developed. They are protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors.

Lamivudine is nucleoside reverse transcriptase inhibitors with activity against human immune deficiency virus (HIV) and hepatitis B virus Fig. 1(a). Nucleoside reverse transcriptase inhibitors (NRTIs) are the prodrugs that require intracellular phosphorylation to their corresponding triphosphate derivatives, which are the active inhibitors of HIV reverse transcriptase. [1-2]

Efavirenz is non-nucleoside reverse transcriptase inhibitors. It is used in the treatment of HIV infection Fig. 1(b). It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme. [3-4]

According to the literature survey it was found that few analytical methods such as UV [5-6] and HPLC methods [7-16] were reported for lamivudine and efavirenz. To our knowledge, no study related to the UV spectrophotometric method for simultaneous estimation of lamivudine and efavirenz have been reported in literature. Therefore, there is a challenge to develop UV Spectrophotometric method for the simultaneous estimation of lamivudine and efavirenz. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical and precise UV

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spectrophotometric method for the simultaneous estimation of two drugs in pharmaceutical dosage form.

MATERIALS AND METHODS

Materials:

Hydrochloric acid, Sodium hydroxide, water, acetonitrile, methanol, lamivudine and efavirenz.

Instrument:

UV double beam spectrophotometer (Shimadzu model 1800) was employed with a spectral band width of 1nm and a wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells)

Method:

The UV spectra's of lamivudine and efavirenz in different solvents like water, acetonitrile, methanol, sodium hydroxide and hydrochloric acid were recorded. These two drugs showed good absorbances when dissolved in acetonitrile. Hence acetonitrile was selected as the solvent for the method. Lamivudine and efavirenz (10 mg each) were separately weighed and transferred to a 100 ml volumetric flask and all the two drugs were dissolved in acetonitrile to get a solution of 100 µg/ml. Working standard solutions of 50 µg/ml of each of the drugs were prepared and scanned in the range 400-200 nm to obtain the absorbance spectra and overlain spectra (Fig.2). Two wavelengths 271 and 247 nm were selected which are the λmax of two drugs lamivudine and efavirenz respectively. The absorbance of lamivudine and efavirenz was measured and the absorptivity values E (1%, 1cm) were determined at all the two selected wavelengths. The concentrations of two drugs in mixture can be calculated using the following equations,

$$C_X = A2ay1 - A1ay2 / ax2ay1 - ax1ay2 \quad \text{.....(1)}$$

$$C_Y = A1ay2 - A2ax1 / ax2ay1 - ax1ay2 \quad \text{.....(2)}$$

where, $C_{lamivudine}$ and $C_{efavirenz}$ are the concentrations of lamivudine and efavirenz respectively in mixture and in sample solutions. A_1 and A_2 are the absorbances of sample at 271 and 247 nm, respectively, ax_1 and ax_2 are the absorptivity of lamivudine at 271 and 247 nm respectively, ay_1 and ay_2 are the absorptivity of efavirenz at 271 and 247 nm, respectively.

RESULTS AND DISCUSSION

Validation of the method:

Linearity:

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 10-100 µg/ml for lamivudine and 10-70 µg/ml for efavirenz. Absorbances were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficients were found to be 0.996 and 0.999 for lamivudine and efavirenz respectively (Fig. 3-4). The results are given in Table 1.

Precision:

To check the degree of repeatability of the method, suitable statistical evaluation was carried out. The concentrations of two drugs were measured three times on the same day at intervals of 1hr and on three different days for intra and inter day study, respectively. The Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated. The results are given in Table 2.

Accuracy:

Recovery studies were carried out by applying the method to drug sample to which known

amount of standard lamivudine and efavirenz corresponding to 50, 100 and 150% of label claim had been added. At each level of the amount six determinations were performed. The results are given in Table 3.

LOD and LOQ:

The LOD of lamivudine and efavirenz was found to be 3.5 µg/ml and 3.0 µg/ml respectively and the LOQ was found to be 10 µg/ml and 10 µg/ml respectively. The results are given in Table 4.

Analysis of marketed formulation:

Twenty Odivir-kits each containing 300 mg lamivudine and 600 mg efavirenz were weighed, average weight was calculated and powdered. A quantity equivalent to 300 mg of lamivudine and 600 mg of efavirenz was weighed and transferred into 100 ml volumetric flask. It is extracted with acetonitrile. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with acetonitrile and filtered. Suitable aliquots of formulation were prepared and scanned to obtain concentration of the two drugs in the linearity range. The concentration of each analyte was determined using the simultaneous equation (Fig. 5) (Table 5).

CONCLUSION

This method is considered simple, reliable, selective providing satisfactory accuracy, precision with lower limits of detection and quantification more specific and sensitive. The good recoveries were obtained in all cases as well as the reliable agreement with the reported procedure proved that the proposed methods could be applied efficiently for determination of lamivudine and efavirenz in oral dosage form with satisfactory precision. More over the shorter duration of analysis for lamivudine and efavirenz makes the reported method suitable for routine analysis in pharmaceutical dosage forms.

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Table 1: Linearity and Correlation coefficient

Parameters	Lamivudine	Efavirenz
Regression equation	y = 0.020x + 0.030	y = 0.057x - 0.004
Linearity µg/ml	10 – 100	10 - 70
Correlation coefficient	0.996	0.999

Table 2: Precision studies

Drug	Concentration µg/ml	Intraday Precision (n=3) % RSD	Inter day Precision (n=3) %RSD
Lamivudine	50	0.487	0.762
Efavirenz	50	0.172	0.356

Table 3: Accuracy

Drug	% Amount added	Amount taken (mg)	Amount recovered (mg)	% Recovery	% *RSD
	50	150	149.42		
Lamivudine	100	300	300.16	99.82	0.461
	150	450	448		
	50	300	299		
Efavirenz	100	600	598.23	99.94	0.546
	150	900	895.14		

*mean of six observations

Table 4: LOD and LOQ studies

Validation parameters	Lamivudine	Efavirenz
Limit of Detection (LOD) µg/ml	3.5	3.0
Limit of Quantification (LOQ) µg/ml	10	10

Table 5: Analysis of Formulation

Drug	Labelled amount (mg/tablet)	Amount found (mg/tablet)	% Label claim	% *RSD
Lamivudine	300	298.92	99.64	0.095
Efavirenz	600	598.67	99.77	0.715

* mean of six observations

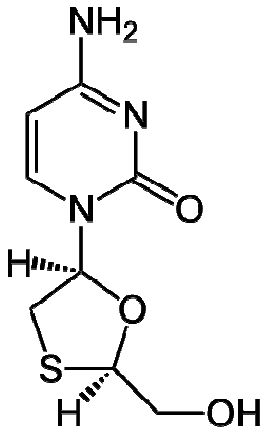

Fig. 1a. Structure of Lamivudine

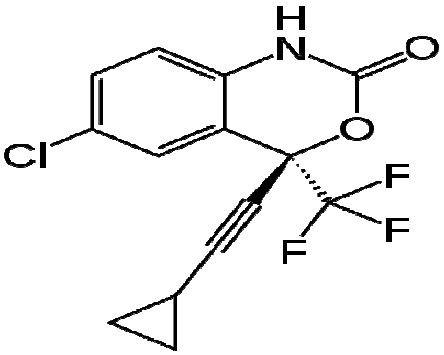

Fig. 1b. Structure of Efavirenz

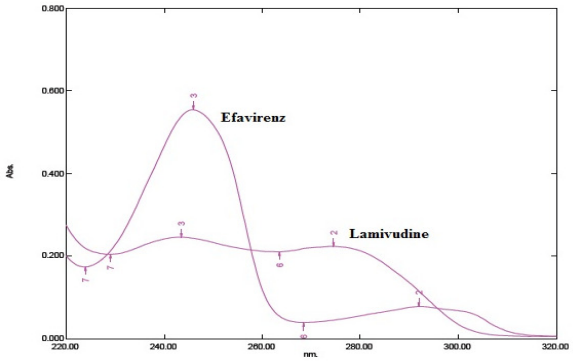

Fig. 2: Overlain Normal spectra of Lamivudine and Efavirenz in Acetonitrile

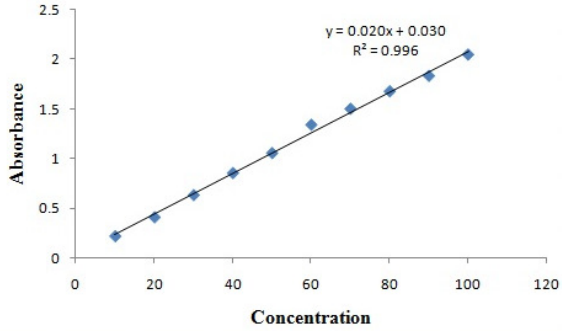

Fig. 3: Calibration Curve of Lamivudine at 271 nm

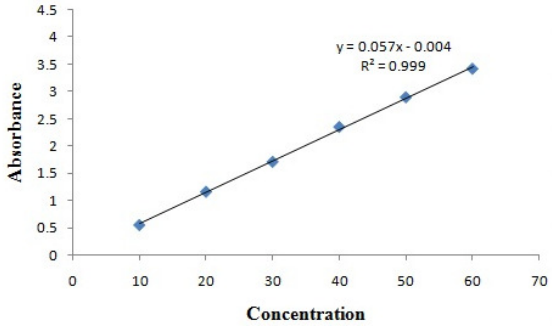

Fig. 4: Calibration Curve of Efavirenz at 247 nm

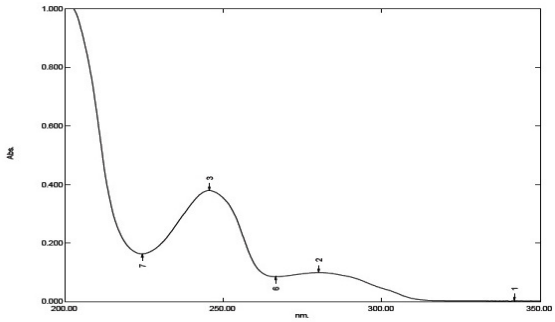

Fig. 5: Spectrum of Lamivudine and Efavirenz formulation

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