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UV Spectrophotometric Absorption Correction Method for the Simultaneous Estimation of Efavirenz, Lamivudine and Zidovudine in Tablet Dosage Forms

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The present paper describes simple, accurate, rapid, precise and sensitive UV spectrophotometric absorption correction method for the simultaneous determination of Efavirenz, Lamivudine and Zidovudine in combined tablet dosage form. Methanol was used as solvent. The wavelengths selected for the analysis using absorption correction method were 305 nm, 250 nm and 254 nm for estimation of Efavirenz, Lamivudine and Zidovudine, respectively. Beer's law obeyed in the concentration range of 4-12 µg/mL, 1-3 µg/mL and 2-6 µg/mL for Efavirenz, Lamivudine and Zidovudine, respectively. The mean percentage drug content for Efavirenz, Lamivudine and Zidovudine were found to be 100%, 99.03% and 98.66%, respectively and the % RSD value was found to be less than 2 which shows the precision of method. The developed method was validated statistically and by recovery studies. The high recovery and low coefficients of variation conforms the suitability of the method for simultaneous analysis of three drugs in combined tablets. Statistical analysis proves that the method was found to be suitable for the routine quality control analysis of Efavirenz, Lamivudine and Zidovudine in pure and pharmaceutical dosage forms.

Keyword: Efavirenz, Lamivudine, Zidovudine, Absorption Correction Method, Validation

1. Introduction

Efavirenz is chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one with the molecular formula $C_{14}H_9ClFNO_2$ with a molecular weight of 315.7g/mol−1.Efavirenz is insoluble in water,soluble in lower alcohol.[2] Lamivudine is a potent synthetic nucleoside analogue with activity against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV). It belongs to chemical class of Pyrimidines and derivatives and is (4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] -1,2-dihydropyrimidin-2-one with molecular formula of $C_8H_{11}N_3O_3S$ and molecular weight of 229.25 g mol−1.Zidovudine is chemically 1- (3-azide-2, 3-di deoxy-β-D-ribofuranosyl)-5-methyl Pyrimidin-2,4(1H, 3H) –dione with the molecular formula C $_{10}H_{13}N_5O_4S$ with a molecular weight of 267.25g/mol−1.[1] Zidovudine is soluble in water,alcohol,acetone,ethanol and sparingly soluble in denatured alcohol.[3]

No methods are reported in literature for the assay of Efavirenz, Lamivudine and Zidovudine in Tablet dosage forms using UV spectrophotometric Absorption correction method. The aim of the study was to develop a simple, precise, accurate, rapid, sensitive and

economic UV spectrophotometric method for the estimation of Efavirenz, Lamivudine and Zidovudine in Tablet dosage forms.

Fig. 1: Structure of Efavirenz

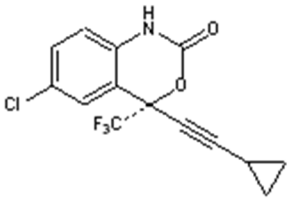


Fig. 2: Structure of Lamivudine

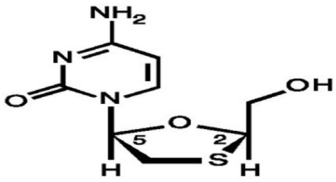
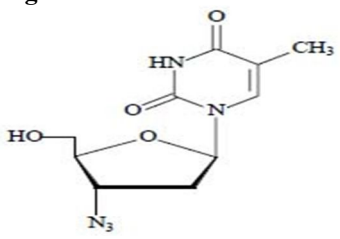


Fig. 3: Structure of Zidovudine



2. Materials and Methods Reagents and Chemicals

Pharmaceutically pure sample of Efavirenz, Lamivudine and Zidovudine were obtained from Mylan Laboratories. Pvt. Ltd., Hyderabad, India.. All chemicals were of analytical grade and supplied by Merck Co, Mumbai, India.

3. Instrumentation

UV spectrophotometric analysis was carried out on Shimadzu 1700 Double beam UV-Vis spectrophotometer, with a pair of 1.0cm matched quartz cells.[2]

4. Experimental condition

According to the solubility characteristics, the common solvent for both the drugs was found to be Methanol. Hence the stock solution was prepared in Methanol and further dilutions were made up with same solvent.

4.1 Preparation of standard stock solution

Accurately weighed quantity 10 mg of each Efavirenz, Lamivudine and Zidovudine were transferred into 10 ml volumetric flask separately. Dissolved in Methanol and diluted to the mark with the same solvent to obtain a standard stock solution 1000 µg/mL of each drug.

4.2 Study of spectral and linearity characteristics

The aliquot portions of standard stock solutions of Efavirenz, Lamivudine and Zidovudine were further diluted with Methanol to get the concentration of 10 µg/ ml of each drug and the solutions were scanned between the range 400 - 200 nm in 1cm cell against blank and the overlain spectra was recorded. From the overlain spectrum of Efavirenz, Lamivudine and Zidovudine in Methanol, it was observed that Lamivudine and Zidovudine have zero absorbance at 305 nm, where as Efavirenz has substantial absorbance. Thus Efavirenz was estimated directly at 305 nm without interference of Lamivudine and Zidovudine. At 250 nm, Zidovudine has zero absorbance. For estimation of Lamivudine, the absorbance of Efavirenz was measured at 250 nm using standard solution of Efavirenz. The contribution of Efavirenz was deducted from the total absorbance of sample mixture at 250 nm. The calculated absorbance was called as corrected absorbance for Lamivudine.[12,13] At 254 nm, these three drugs were showed the absorbance. To estimate the amount of Zidovudine, the absorbance of Efavirenz and Lamivudine were corrected for interference at 254 nm by using absorptivity values. A set of three equations (Equation1, Equation 2 and Equation 3) were framed using absorptivity coefficients at selected wavelengths.

$$Cx = A1/ ax1 \ldots(Eq.\ 1)$$
$$Cy = A2 - ax2\ cx/ay2 \ldots(Eq.\ 2)$$
$$Cz = A3 - (ax2\ cx + ay3cy)/ az3 \ldots(Eq.\ 3)$$

Where, A1, A2 and A3 are absorbance of sample solution at 305nm, 250 nm and 254 nm, respectively.
ax1, ax2 and ax3, absorptivity coefficients of Efavirenz at 305 nm, 250 nm and 254 nm, respectively.
ay2 and ay3, absorptivity coefficients of Lamivudine at 250 nm and 254 nm, respectively.
az3, absorptivity coefficient of Zidovudine at 254 nm.
cx, cy and cz are concentrations of Efavirenz, Lamivudine and Zidovudine respectively in mixture.
For spectrophotometric method, the calibration curves for Efavirenz, Lamivudine and Zidovudine were prepared in the concentration range of 4-12 µg/mL, 1-3 µg/mL and 2-6 µg/mL, respectively at their respective wavelengths by diluting aliquot portions of standard stock solution of each drug.

4.3 Analysis of Tablet Formulation

Twenty tablets were weighed and their mean weight was deteermined. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100mg of Zidovudine was transferred to 10ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and made up to the volume with same. The solution was sonicated for 15minutes and filtered through Whatman filter paper No. 42. An aliquot portion of obtained filtrate was diluted to 10ml with methanol to get final concentration with in linearity range for analysis of Lamivudine and Zidovudine. From the clear solution, further dilution was made to obtain 2.5µg/ml solution of Zidovudine. The absorbance of sample solutions were measured at all selected wavelengths. The content of Efavirenz,[2] Lamivudine and Zidovudine in sample solution of of tablet was calculated. This procedure was repeated for six times.

5. Method Validation

The optimal UV spectrophotometric was completely validated according to the procedure described in ICH guidelines and United State Pharmacopoeia for validation of analytical methods.The performance parameters calculated for the method were linearity, precision, accuracy, limits of detection and quantitation.

Linearity was studied be range of diluting standard stock solution at six different concentrations(n=30 covering the range of 4-12µg/ml, 1-3µg/ml and 2-6µg/ml for Efavirenz, Lamivudine[1] and Zidovudine respectively. Calibration curves with concentration verses absorbance were plotted for three drugs at respective wavelengths and the obtained data were subjected to regression analysis using the least square method.

5.1 Precision

The precision of the method was confirmed by repeatability parameter. The repeatability was performed by the analysis of Formulation for six times with the same concentration. It was expressed as percentage Relative standard deviation (%R.S.D) as series of measurements

5.2 Accuracy

To check the accuracy of the developed methods and to study interferenceon of each of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level. At each level, samples were prepared in triplicate and the mean percentage recoveries and % R S D values were calculated

5.3 Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (4) and (5) respectively. LOD=3.3 δ …. (Eq. 4) LOQ=10 δ ….(Eq.5) where, : standard of y-intercept and S:slope of calibration curve

6. Results and Discussion

An attempt has been made to develop a rapid, sensitive, economic, precise and accurate analytical method for simultaneous estimation of

Efavirenz, Lamivudine and Zidovudine in tablet dosage forms. The proposed method is based on UV spectrophotometric absorption correction method for the simultaneous estimation of Efavirenz,Lamivudine and Zidovudine in UV region using Methanol as solvent. The overlain spectra of Efavirenz, Lamivudine and Zidovudine[3] are shown in Figure 4.

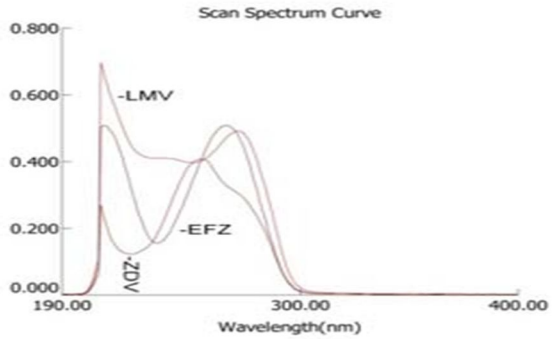


Fig 4: The overlain UV spectra of Efavirenz, Lamivudine and Zidovudine.

6.1 Method validation

Linearity

A linear correlation was found between absorbance and concentrations of drugs. The regression analysis data are represented in **Table 2**. The regression coefficients (r^2) obtained are 0.999,0.999,0.998 for Efavirenz, Lamivudine and Zidovudine which attest the linearity of the method.

Table 1: Regression analysis data

Regression parameters	Efavirenz	Lamivudine	Zidovudine
Concentration range (µg/ml)	4-12	1-3	2-6
Correlation coefficient (r^2)	0.999	0.999	0.998
Slope	0.052	0.092	0.053
Intercept	0.008	0.001	0.004

Precision Mean contents of Efavirenz, Lamivudine and Zidovudine in precision analysis (n=6) were much close to labeled claim of respective drugs. The %R.S.D. value was lower than 2%, assure the precision of the method and the results are shown in **Table2.**

Table 2: Intraday studies

Method	Drug	Label Claim	SD	% RSD
Absorption correction method	Efavirenz	600mg	0.000837	0.1717
	Lamivudine	150mg	0.000837	0.081
	Zidovudine	300mg	0.000837	0.3667

S.D.: Standard deviation; R.S.D.: Relative standard deviation

Table 3: Results of Analysis of Tablet Formulation

Method	Drug	Label claim mg/tab	Estimated amount mg/tab
Absorption correction method	Efavirenz	600mg	8µg/ml
	Lamivudine	150mg	1.9807 µg/ml
	Zidovudine	300mg	3.9460 µg/ml

Accuracy

Accuracy was investigated by means of recovery studies using the proposed method. The percent recoveries after spiking with additional standard drug afford recovery in the range of 98-102% and the results are listed in **Table 4**

Table 4: Result of recovery studies

Drugs	% Amount added	% Recovery(n=3)	±S.D.
Efavirenz	50	99.73	0.8221
	100	99.12	0.6850
	150	99.31	0.5242
Lamivudine	50	99.66	0.6417
	100	99.05	0.5363
	150	99.43	1.0674
Zidovudine	50	98.90	0.1405
	100	99.82	0.7956
	150	99.76	0.7061

S.D.: standard deviation.

LOD and LOQ

The LOD value for Efavirenz, Lamivudine and Zidovudine were found to be 0.08 µg/ml . The LOQ value for Efavirenz, Lamivudine and Zidovudine were found to be 0.2664 µg/ml respectively.

Table 4.: Validation parameters of evaluated method

Parameters	Efavirenz	Lamivudine	Zidovudine
Concentration range (µg/ml)	4-12	1-3	2-6
Intraday Precision (%R.S.D)	0.1717	0.7081	0.3667
Recovery n=9 (±S.D.)	99.39 ± 0.3121	99.38 ± 0.381	99.49 ± 0.5147
LOD (µg/ml)	0.08	0.08	0.08
LOQ (µg/ml)	0.2664	0.2664	0.2664

6.2 Analysis of marketed formulation

The proposed validated method was successfully applied for determination of Efavirenz, Lamivudine and Zidovudine in their Tablet dosage forms. The results of analysis of Tablet dosage form by the proposed method (Table 1), expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets.

7. Conclusion

UV spectrophotometric absorption correction method was developed and validated for the determination of Efavirenz, Lamivudine and Zidovudine. The developed method was found to be simple, specific, rapid, precise and accurate from the results of validation parameters. Hence the proposed method could be effectively applied for the routine quality control analysis of Efavirenz, Lamivudine and Zidovudine in bulk and pharmaceutical dosage form.

8. Acknowledgments

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