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Simultaneous equation method for the estimation of Lamivudine, Nevirapine and Zidovudine in Bulk and tablet dosage forms

***Ramesh Jayaprakash, Senthil Kumar Natesan, Vijay Amirtharaj Ramasamy, Rajasekhar Kommi, Kiran Gandhi R.**

Department of Pharmaceutical Analysis, JKK Munirajah Medical Research Foundation's- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam-638183, TN, India

Abstract: A simple, accurate, precise, economical and reproducible UV-Visible Spectrophotometric method has been developed for the simultaneous estimation of Lamivudine, Nevirapine and Zidovudine in bulk and in combined tablet dosage form. The stock solutions were prepared in methanol followed by the further required dilutions with distilled water. This method involves the formation and solving of simultaneous equations at 271, 282 and 267 nm, as absorbance maxima of lamivudine, nevirapine and zidovudine, respectively. Beer's law obeyed the concentration range of 1.5-9, 2.5-15 and 3-18 µg/mL for Lamivudine, Nevirapine and Zidovudine, respectively. The results of analysis were validated statistically and by recovery studies. The %RSD for the recovery study was less than 2. The proposed method can be effectively applied for the simultaneous estimation of these drugs in bulk and in combined tablet dosage form.

Keywords: Lamivudine; Nevirapine; Zidovudine; UV-Visible Spectrophotometry; simultaneous equation method

1. Introduction

These drugs active against human immunodeficiency virus (HIV) which is a retro virus.[1] They are useful in prolonging and improving the quality of life and postponing complications of acquired immune deficiency syndrome (AIDS) or AIDS-related complex (ARC), but do not cure the infection. The clinical efficiency of anti-retroviral drugs is monitored primarily by plasma HIV-RNA assays and CD4 lymphocyte count carried out at regular intervals.[2] Estimation of anti-retroviral drugs in combinations dosage forms by UV-Visible,[3-5] Visible,[6] RP-HPLC,[3, 7, 8] HPTLC[3, 9-11] and in human plasma by an ion-pair HPLC[12] and HPTLC[13] method were reported. The current paper discusses simultaneous equation method for the estimation of Zidovudine (ZID), Nevirapine (NEV) and Lamivudine (LAM) in tablet dosage forms.

Zidovudine(ZID): It is a thymidine analogue (azido thymidine, AZT, **Figure 1**), the prototype nucleoside reverse transcriptase inhibitor (NRTI). After phosphorelation in the host cell, zidovudine triphosphate selectively inhibits viral reverse transcriptase (RNA dependant DNA polymerase) in preference to cellular DNA polymerase.[14]

Nevirapine(NEV): It is nucleoside unrelated compound which directly inhibit HIV reverse transcriptase without the need for intracellular phosphorelation (**Figure 1**). Its locus of action on the enzyme is also different. It is more potent than AZT on HIV-1, but do not inhibit HIV-2.[15]

Lamivudine(LAM): This deoxycytidine analogue is phosphorelated intracellularly and inhibit HIV reverse transcriptase as well as hepatitis-B virus (HBV) DNA polymerase (**Figure 1**). Its incorporation into DNA results in chain termination.[2]

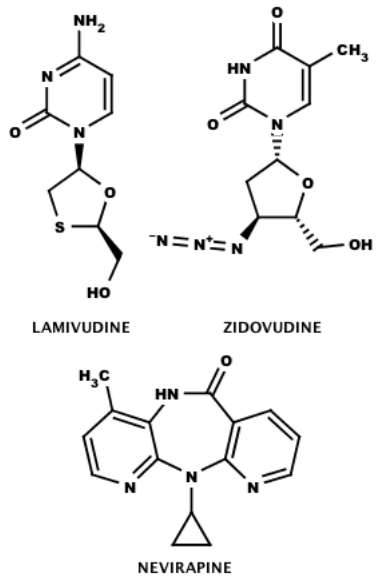

Figure 1. Structure of the drugs

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*corresponding author: Initial Tel: +91-9042345272;
E-mail: rameshj1974@gmail.com

2. Results and Discussion:

The proposed method is based on spectrophotometric simultaneous estimation of LAM, NEV and ZID in UV region using methanol and distilled water as solvent. The method developed and validated according to the ICH guidelines. The overlain spectra showed the maximum absorbance at 271nm for LAM, 282nm for NEV and 267nm for ZID (**Figure 2**). This was involves in the construction and solving of simultaneous equations using absorptivity coefficient values.

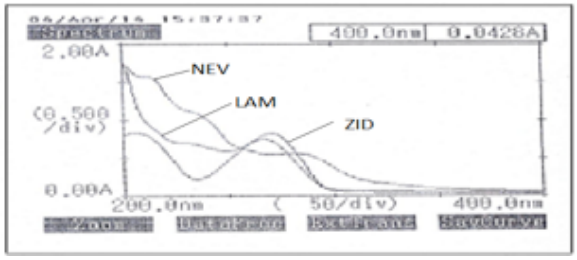

Figure 2. Overlay UV spectrum of Lamivudine, Nevirapine and Zidovudine

The linearity was determined for three drugs by measuring the different concentrations range of 1.5-9 µg/mL of LAM, 2.5-15 µg/mL of NEV and 3-18 µg/mL of ZID at their respective wavelengths of 271, 282 and 267nm, respectively. By using the absorbance values the calibration graph was plotted (absorbance Vs concentration) **Figure 3.1-3.3**, from the calibration curve calculated the correlation co-efficient values were found to be 0.999, 0.999 and 0.998 for LAM, NEV and ZID, respectively. The optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept, sandelle's sensitivity and molar absorptivity were calculated and are summarized in **Table 1**.

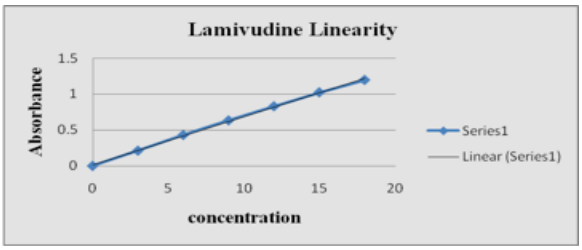

Figure 3.1 Standard curve linearity for Lamivudine

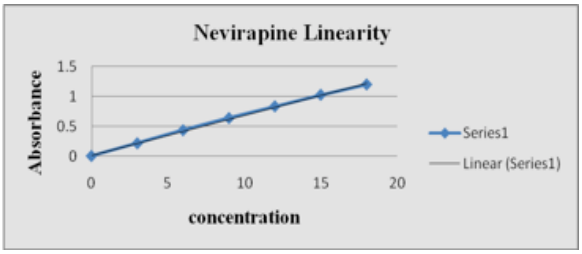

Figure 3.2 Standard curve linearity for Nevirapine

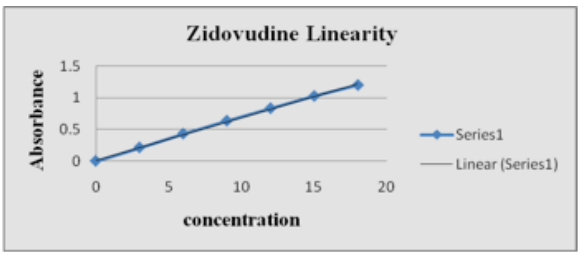

Figure 3.3 Standard curve linearity for Zidovudine

The LOD and LOQ values were found to be 0.1460, 0.1115 and 0.1838, 0.4425, 0.3381 and 0.5572 µg/mL for LAM, NEV and ZID, respectively. The values indicate the sensitivity of the simultaneous equation method.

Table 1. Optical and Regression characteristics of Lamivudine, Nevirapine and Zidovudine

Parameter	LAM (271nm)	NEV (282nm)	ZID (267nm)
Beer's law limit (µg/ mL)	1.5-9	2.5-15	3-18
Molar absorptivity	13656.0038	10943.3135	18377.3574
Sandell's sensitivity (µg/cm²/0.001 abs unit)	0.01690	0.0249	0.0149
Regression equation (y=a+bc)	0.0591	0.0400	0.0669
Slop (b)			
Intercept (a)	0.0037	0.0091	0.0178
Correlation coefficient (r)	0.9994	0.9995	0.9986
Standard Error	0.0010	0.0004	0.0028
LOD	0.1460	0.1115	0.1838
LOQ	0.4425	0.3381	0.5572

The percentage label claim present in tablet formulation was found to be 99.10±0.6571, 99.43±0.5248 and 99.85±0.2036for LAM, NEV and ZID, respectively (**Table 2**). Data for the recovery studies were presented in **Table 3**. Precision of the method was confirmed by the repeated analysis of formulation for six times. The % RSD values were found to be 0.6626, 0.5278 and 0.2039 for LAM, NEV and ZID, respectively. The low %RSD values indicated that all the three drugs showed good agreement with the label claim ensures the precision of method (**Table 4**). Further, the precision of the method was confirmed by system precision, repeatability; inter day and intra day precision. The % RSD values were calculated and values were shown in **Table 4**.

Table 2. Results of analysis of tablet formulation

Drug	Amount (mg/tablet)		%Drug content	SD	% RSD
	Label	Estimated			
LAM	30	29.73	99.10	0.6567	0.6626
NEV	50	49.71	99.43	0.5248	0.5278
ZID	60	59.91	99.85	0.2036	0.2039

Acceptance criteria: The %RSD should be less than 2%

Table 3. Recovery studies

Drugs	% Soln	Amt (µg)		% Recovery	[a]SD	[a]%RSD
		Add	Rec			
LAM	80	2.4	2.38	99.16	0.5851	0.5867
	100	3	3.01	100.33		
	120	3.6	3.59	99.72		
NEV	80	4	3.96	99.00	0.9328	0.9346
	100	5	4.98	99.60		
	120	6	6.05	100.83		
ZID	80	4.8	4.78	99.58	0.1253	0.1256
	100	6	5.99	99.83		
	120	7.2	7.18	99.72		

[a]Mean (n=3); Add-Added; Rec-Recovered; Acceptance criteria: The percentage recovery should be in between 98-102% & The %RSD should <2.

Table 4. Precision

Parameters	Mean±%RSD		
	LAM	NEV	ZID
System precision	0.1855±0.0811	0.2152±0.0992	0.4307±0.0228
Repeatability	98.90±1.0242	99.43±0.7581	99.85±0.1692
Intraday precision	0.5544±1.1976	0.6737±0.7348	0.9852±0.4607
Inter day precision	0.5581±1.0400	0.66±0.3239	0.9877±0.2463

Acceptance criteria: The %RSD should be less than 2%.

The accuracy of the method was performed by recovery studies by using the 80%, 100% and 120% sample solution containing their respective concentrations. By using these solutions absorbance was recorded at 271nm, 282nm and 267nm. Calculate the amount found and % of amount recovered, the values were shown in **Table 3**.

3. Experimental

3.1. Instrumentation:

The present work was carried out on Shimadzu-1700 double beam UV-Visible spectrophotometer with pair of 10mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulfuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

3.2. Reagents and chemicals

Pharmaceutically pure sample of LAM, NEV and ZID were obtained as a gift sample from Mylan Laboratories, Pudhucherry. All solvents were of analytical grade obtained from High pure laboratories, Mumbai, India. A combination of LAM (30 mg), NEV (50 mg) and ZID (60 mg) in tablet formulation was obtained from Mylan Laboratories, Pudhucherry, India.

3.3. Experimental conditions

According to the solubility characteristics, the common solvents for three drugs were found to be methanol. Hence the stock solution was prepared in methanol and further dilutions were made up with double distilled water.

3.4. Preparation of standard stock solution and selection of wavelength

Pure raw materials of 3mg of LAM, 5mg of NEV and 6mg of ZID was taken in a three separate 100 mL volumetric flasks, to that 10 mL of methanol was added to dissolve and made up the volume to 100 mL with distilled water. 1 mL of each above stock solution was taken in to separate 10 mL volumetric flasks and make up the volume with water to get the concentration of 3, 5 and 6 µg/mL of LAM, NEV and ZID, respectively.

Standard stock solutions of 10 µg/mL of LAM, 10 µg/mL of NEV and 10 µg/mL of ZID were prepared. The spectrum of these solutions was recorded by scanning in the spectrum mode between 200-400 nm, against methanol and distilled water as blank solution. From the spectrum it was found that LAM, NEV and ZID shown the maximum absorbance at 271, 282 and 267 nm respectively, and by using memory channels, the three spectra's were overlapped.

3.5. Study of spectral and Linearity characteristics

The above standard stock solutions were scanned between the range 200-400 nm in 1 cm cell against distilled water as blank and the overlain spectra was recorded.

In quantitative estimation of three compounds by simultaneous equation method, three wavelengths i.e. 271 nm , λ_{max} of LAM, 282 nm, λ_{max} of NEV and 267 nm, λ_{max} of ZID, were selected from the overlain spectra and at which all the three drugs have absorbance at all others using absorptivity coefficients at selected wavelengths. The concentration of three drugs in the mixture was calculated using the following equations.

$$x = \frac{A1[b3c2 - b2c3] + A2[b1c3 - b3c1] + A3[b2c1 - b1c2]}{[a3b2 - a2b3]c1 + [a1b3 - a3b1]c2 + [a2b1 - a1b2]c3}$$

$$y = \frac{A1[a2c3 - a3c2] + A2[a3c1 - a1c3] + A3[a1c2 - a2c1]}{[a3b2 - a2b3]c1 + [a1b3 - a3b1]c2 + [a2b1 - a1b2]c3}$$

$$z = \frac{A1[a3b2 - a2b3] + A2[a1b3 - a3b1] + A3[a2b1 - a1b2]}{[a3b2 - a2b3]c1 + [a1b3 - a3b1]c2 + [a2b1 - a1b2]c3}$$

Where;

a1, a2 and **a3** = absorptivity values of compound '**a'** at λ_1, λ_2 and λ_3 respectively.

b1, b2 and **b3 =** absorptivity values of compound '**b'** at λ_1, λ_2 and λ_3 respectively.

c1, c2 and **c3 =** absorptivity values of compound **'c'** at λ_1, λ_2 and λ_3 respectively.

A1, A2 and **A3** = absorbance of diluted sample at λ_1, λ_2 and λ_3 respectively.

x, y and **z =**concentration of LAM, NEV and ZID.

Further dilutions are made from stock solution to get the concentration ranging from 1.5-9 µg/mL of LAM, 2.5-15 µg/mL of NEV and 3-18 µg/mL of ZID respectively. The absorbances of these solutions were measured and the calibration graphs were constructed. The optical characteristics are shown in **Table 1**. The calibration graphs are shown in **Figure 3.1 - 3.3**.

3.6. Analysis of Tablet formulation

Weighed 20 tablets and calculated the average weight, weight to be taken was calculated crushed the tablets and the powder equivalent to 3,5 and 6mg of LAM, NEV and ZID was taken in to a 100mL volumetric flask and added 10mL of methanol to dissolve and final volume was made with water. Sonicated the solution for 15 min and filtered the solution through whattman filter paper. 1mL of the above resulting solution was taken and transfers in to a 10mL volumetric flask and made up the volume with water to get the concentration of 3, 5 and 6 µg/mL of LAM, NEV and ZID respectively.

The formulation of LAM, NEV and ZID was estimated by using the simultaneous equation method. In this method first baseline was corrected by using blank solution and the absorbance of the sample was recorded at 271, 282, 267 nm.

All the absorbance values were converted to absorptivity values and include these values in simultaneous equation and calculated the concentration of the three drugs, and calculated the amount present in the formulation (mg/tablet) the percentage of amount present in the formulation and SD and % RSD values were calculated.

3.7. Validation of methods

The method was in compliance with ICH guidelines (ICH, Q2 (R1), 2005). The following parameters were used for validation of the developed method.

3.7.1. Linearity

Adequate dilutions are made from stock solution to get the concentration ranging from 1.5-9 µg/mL of LAM, 2.5-15 µg/mL of NEV and 3-18 µg/mL of ZID respectively. These solutions were scanned at their respective wavelengths and recorded the absorbance of individual standards and the results were calculated**,** the calibration graph was plotted.

3.7.2. Accuracy

Recovery studies of the drugs were carried out for accuracy. It was prepared by mixing a known quantity of standard drug with the pre-analyzed sample formulation and the contents were re-analyzed by proposed method. This was carried out at 80%, 100% and 120% levels. The percentage recovery and %RSD was calculated.

3.7.3. Precision

System precision: Standard solutions were prepared by adding 3, 5 and 6mg of standard LAM, NEV and ZID in to a 100mL volumetric flask, methanol was added to dissolve and made up to the volume to 10 mL with water

1mL from the above solution was pipette out and transferred in to a 10mL volumetric flask and made up the volume with water to get the concentration of 3, 5 and 6 µg/mL. The absorbance of the solutions was measured for six times and the SD and %RSD values for three drugs are calculated, the value should not more than 2%.

Method precision: The sample solution was prepared as per the procedure followed in the estimation of the formulation and the resulting solution containing the concentration 3, 5 and 6 µg/mL of LAM, NEV and ZID were scanned at 200-400nm and the absorbance was recorded at 271nm, 282nm and 267nm. The amount present in the sample was calculated by using simultaneous equation. The SD and %RSD was calculated.

Intra day precision and Inter day precision: Sample solution was prepared having the concentration of 3, 5 and 6µg/mL of LAM, NEV and ZID, respectively. Record the absorbance of the solution at different wavelengths is 271nm, 282nm and 267nm at different time intervals (0, 3 and 6hrs) in the same day by using same concentration solution. The SD and %RSD was calculated.

In inter day precision method the sample solution was scanned at three different days (Day1, Day2 and Day3) intervals by using same concentration and calculated the SD and %RSD values.

Abbreviation

LAM-Lamivudine; NEV-Nevirapine; ZID-Zidovudine

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