RASĀYAN *J. Chem.*
Vol. 7 | No.1 | 80-87 | January - March| 2014
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

MATRIX METHODOLOGY FOR MULTICOMPONENT PHARMACEUTICAL DOSAGE FORM

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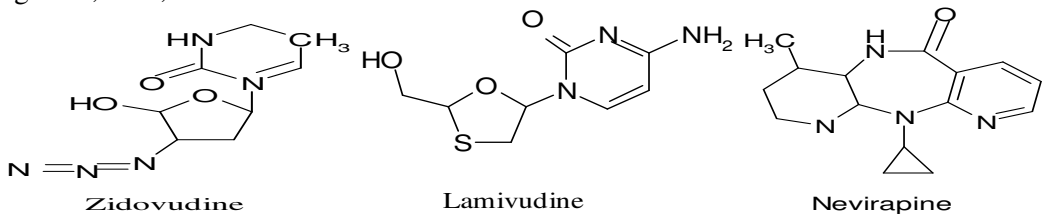
ABSTRACT
The Multiwavelength spectrophotometric methods, TLRC (Tri linear regression analysis), MLRC (Multi linear regression analysis), and CLS (classical least analysis) were developed for Lamivudine Zidovudine and Nevirapine having overlapped spectra in the region of 200-400nm. In these methods nine wavelengths were selected for the estimation of these drugs. The matrix was set up for all three methods for the quantitative estimation of the drugs in their solution respectively and the recovery study was carried out for the commercial tablet formulation of two batches. The developed matrix methodology is one of the methods to increase accuracy of analysis by UV-Spectrophotometer using linear regression analysis method.
Keywords: Tri linear regression analysis; Multi linear regression analysis; classical least square analysis; Multiwavelength ; Lamivudine; Zidovudine; Nevirapine.

INTRODUCTION

Retroviral infections are treated with antiretroviral drugs. Maximum three drugs in combination are used to reduce retroviral infection in HIV infected patients.[1] The combination treatment is effective in first line therapy, includes nucleoside reverse transcriptase inhibitor, protease inhibitor and non nucleoside transcriptase inhibitor.[2]Chemically Zidovudine(zido) is 1-(3-azido-2,3-dideoxy-β-$_D$-ribofuranosyl)-5-methyl pyrimidine-2,4(1H,3H)-dione[3] ; Lamivudine (Lam) is (2R,5S)-4amino-1-[2-hydroxymethyl-1,3-oxathion-5yl]-2(1H)-pyrimidinone[3,4], Nevirapine (Nevi) is 11Cyclopropyl-5,11-dihydro-4methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one[3,4]. The active triphosphates metabolites of Lamivudine and Zidovudine act against HIV by inhibition of reverse transcriptase Via DNA chain termination after incorporation of the nucleotide analogue[5]. Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site[5,6]. The activity of Nevirapine does not compete with template or nucleoside triphosphates[6].

According to the literature survey the various analytical methods have been developed for the determination of these drugs by RP-HPLC[1,2,7,8,] and HPTLC-Densitometric method[9], Bioanalytical method using HPLC-UV[10], LC and ion pair HPLC.

The aim of the proposed study was to develop and validate UV spectrophotometric method for quantification of Zido, Lam, and Nevi in pure and tablet dosage form simultaneously by multiwavelength linear regression analysis.[11-20] The validated method was applied to analysis of the tablet dosage form containing Zido, Lam, and Nevi.



EXPERIMENTAL

Instruments

A Shimadzu UV - 1650 PC double beam UV-Visible spectrophotometer with1cm path length supported by Shimadzu UV-Probe software, version 2.21 was used for the spectral measurement.

Chemicals

Zidovudine Lamivudine and Nevirapine pure samples were obtained from micro lab Bangalore as a gift samples. Methanol used was of analytical grade.
Commercial tablet formulation Duovir-N® tablet Cipla LTD., two batch no-X30264 and X3065 consisting of Lamivudine 150 mg, Zidovudine 300 mg and Nevirapine 200 mg per tablet. Tablet was purchased from local market.

Standard stock solution

Stock solution of 10mg/100ml Zido, Lam and Nevi were prepared in 20:80 Methanol water. Further dilutions were prepared from 2- 10µg/ml for individual drug. All the solutions were prepared freshly.

Sample stock solution

Ten tablets were accurately weighed and powdered in mortar. An amount equivalent to 300mg of Zido was dissolved in 20:80 Methanol water in 100 ml of volumetric flask with the aid of sonication for 15 min. The solution was filtered using whatman filter paper.

Methodology

Zido, Lam and Nevi standard stock solution were used for development of validated UV method at individual wavelengths as per ICH guidelines.

Linear regression analysis

TLRC method

For TLRC method three wavelengths were considered for the analysis of the component mixture [Zido(X), Lam (Y), Nevi (Z)].The three linear regression equations were obtained by using the absorbance measured at three wavelengths against the concentrations of standard solution for each component. The slope values obtained from the linear regression analysis for each component were used for the formation of the matrix set. The wavelengths selected for the analysis were 266nm, 271nm, 282nm.

Equations for the formation of matrix are-

$$A_{mix1}=b_{x1}C_x+b_{y1}C_y+b_{z1}C_z+a_{xyz1}$$
$$A_{mix2}=b_{x2}C_x+b_{y2}C_y+b_{z2}C_z+a_{xyz2}$$
$$A_{mix3}=b_{x3}C_x+b_{y3}C_y+b_{z3}C_z+a_{xyz3}$$

Where, A_{mix1}, A_{mix2} and A_{mix3} are the absorbance of the mixture of X, Y, Z analytes at three wavelengths set. a_{xyz1}, a_{xyz2} and a_{xyz3} are the sum of intercepts of the linear regression equation at the three wavelengths
Conversion of equation into matrix form-

$$\begin{pmatrix} A_{mix1} - axyz_1 \\ A_{mix2} - axyz_2 \\ A_{mix3} - axyz_3 \end{pmatrix} = \begin{pmatrix} bx_1 & by_1 & bz_1 \\ bx_2 & by_2 & bz_2 \\ bx_3 & by_3 & bz_3 \end{pmatrix} \times \begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix}$$

MLRC method

The method is similar to TLRC method, but MLRC method contains more wavelengths procedure instead of three wavelengths. For this reason nine wavelengths (nm) selected (256, 261, 266, 271, 276, 282, 287, 292 and 297).

Equations for matrix formulation are-

$A_{mix1}=b_{x1}C_x+b_{y1}C_y+b_{z1}C_z+a_{xyz1}$

$A_{mix2}=b_{x2}C_x+b_{y2}C_y+b_{z2}C_z+a_{xyz2}$

....

$A_{mix9}=b_{x9}C_x+b_{y9}C_y+b_{z9}C_z+a_{xyz9}$

Equation converted into matrix form-

$(A_{mix} - a_{xyz})_{9\times1} = K_{9\times3}\, C_{3\times1}$

The matrices, $(A_{mix} - a_{xyz})_{9\times1}$ and $K_{9\times3}\, C_{3\times1}$ are multiplied by the transpose$(K')_{9\times3}$ of the matrix $K_{9\times3}$. Written as,

$C_{3\times1}= [(K')_{3\times n}\, K_{9\times3}]^{-1}{}_{3\times3} \times [(K')_{3\times9} \times (A_{mix} - a_{xyz})_{9\times3}]$

CLS method

This method is based on the absorptivity values at selected wavelengths for spectrophotometric estimation. In this method nine wavelength were selected for the analysis. Selected wavelengths (nm) are (256, 261, 266, 271, 276, 282, 287, 292, and 297)

Equations for matrix formation-

$A_1=\alpha_1Cx+\beta_1Cy+\gamma_1Cz$

$A_2=\alpha_2Cx+\beta_2Cy+\gamma_2Cz$

… …. ….. ….

$A_9= \alpha_9Cx+\beta_9Cy+\gamma_9Cz$

Where,

A_1, A_2….A_9 represent absorbance 0f solution of mixture X, Y, Z component.

$\alpha_{1,2,3}\ldots\alpha_9$, $\beta_{1,2,3}\ldots\beta_9$, $\gamma_{1,2,3}\ldots\gamma_9$ represent absorbtivity values calculated for X,Y,Z respectively , at $\lambda_{1,2,3}\ldots\lambda_9$ wavelengths.

Equation converted into matrix form-

$$\begin{pmatrix} A_1 \\ A_2 \\ \ldots \\ A_9 \end{pmatrix} = \begin{pmatrix} \alpha_1 & \beta_2 & \gamma_3 \\ \alpha_1 & \beta_2 & \gamma_3 \\ \ldots & \ldots & \ldots \\ \alpha_9 & \beta_9 & \gamma_9 \end{pmatrix} \times \begin{pmatrix} CX \\ CY \\ CZ \\ \end{pmatrix}$$

The CLS method is solved using similar procedure as that of MLRC method.

RESULTS AND DISCUSSION

The Duovir-N® a popularly used formulation to treat retroviral infection is tricomponent dosage form consists of Zido, Lam, and Nevi As the spectra of Zido, Lam and Nevi in mixture showed overlapping it is difficult to determine simultaneously all three using conventional UV- Spectrophotometric method. The TLRC, MLRC and CLS methods were developed for accurate and precise analysis of all three in combination by spectrophotometric methods without physical separation of the component. The individual spectra of Zido (λ_{max}=266nm) Lam (λ_{max}=271nm) and Nevi (λ_{max}=282nm) and their mixture were recorded from λ_{max} 200- 400nm (fig.-1).

TLRC method

The absorbance of each drug was recorded at three wavelengths 266, 271 and 282(nm) from 2-10 µg/ml. the linear regression equations were obtained by calibration curve at all the wavelengths.

Equation for TLRC method-

λ_1 =266, A_{mix1}

$=0.037_{(Zido)} + 0.042_{(Lam)} + 0.028_{(Nevi)}$

λ_2=271, A_{mix2}

$= 0.035_{(Zido)} + 0.045_{(Lam)} + 0.027_{(Nevi)}$

λ_3=282, A_{mix3}

$= 0.020_{(Zido)} + 0.031_{(Lam)} + 0.028_{(Nevi)}$

Matrix sets -

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 0.042 & 0.037 & 0.028 \\ 0.045 & 0.035 & 0.027 \\ 0.031 & 0.020 & 0.028 \end{pmatrix}^{-1} \times \begin{pmatrix} 0.091 \\ 0.091 \\ 0.066 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 23.80 & 27.02 & 35.71 \\ 22.22 & 28.57 & 37.03 \\ 32.25 & 50 & 35.71 \end{pmatrix} \times \begin{pmatrix} 0.091 \\ 0.091 \\ 0.066 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 2.16 & 2.45 & 2.35 \\ 2.02 & 2.59 & 2.44 \\ 2.93 & 4.55 & 2.35 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 6.96 \\ 7.05 \\ 9.83 \end{pmatrix}$$

MLRC method

The absorbances for the three drugs were recorded at nine wavelengths from 2-10 µg/ml. The linear regression equations were obtained by calibration curve at all the nine wavelengths.

Equations for MLRC method-

λ_1=256 $Amix_1$

$= 0.033+ 0.029+0.032$

λ_2=261 $Amix_2$

$= 0.039+0.034+S0.030$

λ_3=266 $Amix_3$

$= 0.042+0.037+0.028$

λ_4=271 $Amix_4$

$= 0.045+0.035+.0.027$

λ_5=276 $Amix_5$

$= 0.043+0.030+0.028$

λ_6=282 $Amix_6$

$= 0.031+0.020+0.028$

λ_7=286 $Amix_7$

$= 0.019+0.012+0.028$

λ_8=292 $Amix_8$

$= 0.006+0.005=0.025$

λ_9=297 $Amix_9$

$= 0.001+0.001+0.021$

Matrix sets-

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 0.0174 & 0.00746 & 0.009385 \\ 0.00746 & 0.006358 & 0.006277 \\ 0.009385 & 0.006277 & 0.006855 \end{pmatrix}^{-1} \times \begin{pmatrix} 0.0177 \\ 0.0168 \\ 0.0126 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 57.47 & 134.04 & 106.55 \\ 134.04 & 157.28 & 159.31 \\ 106.55 & 159.31 & 145.87 \end{pmatrix} \times \begin{pmatrix} 0.0177 \\ 0.0168 \\ 0.012 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 1.09 & 2.26 & 1.34 \\ 1.8 & 2.65 & 2.01 \\ 1.8 & 2.6 & 1.7 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 4.6 \\ 6.4 \\ 6.1 \end{pmatrix}$$

CLS method

The absorbances for the three drugs were recorded at nine wavelengths from 2-10 µg/ml. The absorbtivity values were calculated at nine wavelengths for the individual drugs.

Matrix sets-

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 11204827.26 & 11192884.02 & 277813.924 \\ 11192884.02 & 11182637.47 & 273878.737 \\ 277813.749 & 273878.7378 & 278477.69 \end{pmatrix}^{-1} \times \begin{pmatrix} 598.36715 \\ 575.11969 \\ 266.29449 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 0.0000000892 & 0.0000000893 & 0.000003599 \\ 0.0000000893 & 0.0000000894 & 0.000003651 \\ 0.00000359 & 0.000003651 & 0.000003590 \end{pmatrix} \times \begin{pmatrix} 598.36715 \\ 575.11969 \\ 266.29449 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 0.000053 & 0.000051 & 0.00095 \\ 0.000053 & 0.000051 & 0.00097 \\ 0.00215 & 0.00209 & 0.0095 \end{pmatrix}$$

$$\begin{pmatrix} CX \\ CY \\ CZ \end{pmatrix} = \begin{pmatrix} 0.00100 \\ 0.00107 \\ 0.00113 \end{pmatrix}$$

Concentration of drugs obtained in g/100ml.

Table-1: Linear regression analysis and its results at nine wavelengths

λ max	Zidovudine		Lamivudine		Nevirapine	
	Linear equation	R	Linear equation	r	Linear equation	R
256	A=0.033+0.010	0.997	A=0.029+0.001	0.998	A=0.032-0.000	0.999
261	A=0.039+0.002	0.996	A=0.034+0.004	0.997	A=0.030-0.003	0.999
266	A=0.042+0.015	0.997	A=0.037+0.002	0.999	A=0.028-0.001	0.999

271	A=0.045+0.015	0.997	A=0.035+0.002	0.999	A=0.027-0.001	0.999
276	A=0.043+0.012	0.985	A=0.030+0.002	0.999	A=0.028-0.000	0.999
282	A=0.031+0.010	0.997	A=0.020+0.000	0.999	A=0.028-0.003	0.998
287	A=0.019+0.005	0.997	A=0.012-0.000	0.999	A=0.028-0.001	0.999
292	A=0.006+0.000	0.997	A=0.005-0.001	0.999	A=0.025-0.000	0.999
297	A=0.001-0.001	0.978	A=0.001-0.001	0.989	A=0.021-0.000	0.999

A= absorbance 0f compound, r = correlation coefficient

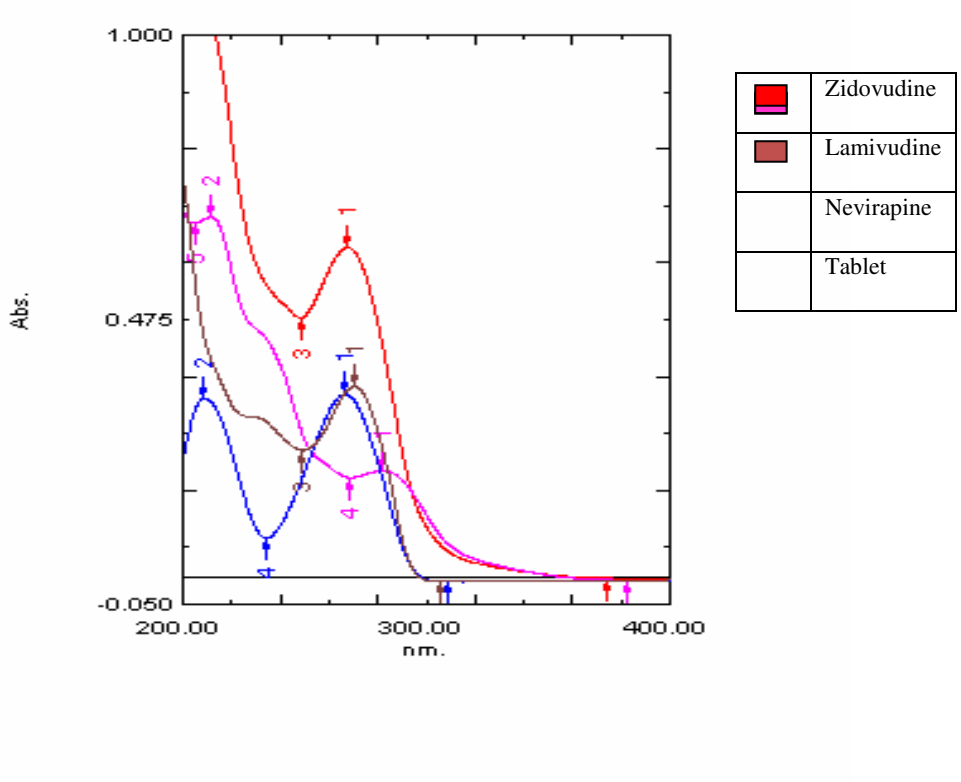


Fig.-1: Absorbance spectra of: Zido 10 µg/ml; Lam 10 µg/ml; Nevi 10 µg/ml; Tablet 10 µg/ml in methanol and water (20:80).

Table-2: Absorptivity values calculated for CLS method

S.No.	λ max	Lamivudine	Zidovudine	Nevirapine
1	256	37.59	39.06	46.72
2	261	31.74	33.89	50.50
3	266	54.34	29.67	54.34
4	271	31.05	28.32	54.64
5	276	37.03	30.86	52.91
6	282	55.24	42.19	51.28
7	287	99	71.42	52.91
8	292	277.77	243.90	59.52
9	297	3333.33	3333.33	73.52

Table-3: Result obtained from the developed methods

Methods	Lamivudine	Zidovudine	Nevirapine
	Mean±SD	Mean±SD	Mean±SD
TLRC	6.62±0.17	6.4±0.4	8.9±0.7
MLRC	5.28±0.5	5.82±0.8	7.8±1.5
CLS	9.8±2.2	9.7±1	10.8±1.1

The results obtained are average of three experiments; SD = Standard deviation

Table-4: Recovery study for determination of Zido Lam and Nevi in pharmaceutical dosage form of two batches

Conc. µg/ml	Batch 1 % Recovery			Batch2 % Recovery		
	Zido	Lam	Nevi	Zido	Lam	Nevi
1	104.43	104.97	103.88	101.9	102.48	106.84
1.5	100.38	99.99	101.37	97.76	96.90	97.48
2	108.41	108.72	111	96.18	97.68	97.48
2.5	97.73	97.65	95.27	97.44	97.56	97.78
3	102.99	101.73	108.23	99.47	99.56	101.37

The above results obtained are mean of three experiments, Batch 1: Duovir-N (X30264) batch 2: Duovir-N (X30265).

CONCLUSION

In the proposed work the three UV-Spectrophotometric methods were developed for the multicomponent pharmaceutical dosage form containing Lam, Zido, and Nevi. The individual spectra of these three drugs showed overlapping in UV region. Therefore the matrix method was developed for simultaneous analysis of the zido lam and nevi. The methods was developed using the linear regression analysis. All three methods increased accuracy for simultaneous analysis of three components in their mixture and pharmaceutical dosage form. The recovery study was done by standard addition method for the pharmaceutical dosage form, satisfactory result obtained for both the batches. The above methods can be applied for the routine analysis. Methods are applicable for Quality control of multicomponent formulation.

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[RJC-1106/2014]