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Simultaneous Assay Of Lamivudine And Zidovudine In Combination Tablets By Derivative Spectrophotometry

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ABSTRACT

A simple and rapid method based on derivative spectroscopy has been proposed for simultaneous estimation of Lamivudine and Zidovudine in multicomponent formulations. Each drug is estimated at wavelengths where other show zero amplitude in first derivative spectra. The condition of derivatization was optimized. Proper selections of wavelengths eliminate complex interference raised in estimation of one drug by other. The results of analysis have been validated statistically at a 95 percent confidence level with student's t test and by recovery studies. No interference was observed from common excipients.

KEY WORDS: *Derivative spectroscopy; Zero crossing; Compensation technique*

INTRODUCTION:

Lamivudine, 2′-deoxy-3′-thiacytidine (3TC) a pyrimidine analog is an oral medication, used for the treatment of infections with the human immunodeficiency (HIV) and hepatitis B viruses. It works by stopping the spread of the HIV and hepatitis B viruses. Zidovudine (azidothymidine, AZT), 3′-azido-3′deoxythymidine, a synthetic pyrimidine nucleoside analogue of the naturally occurring nucleoside thymidine, is an anti-HIV medication, available in capsule, tablet, and syrup formulations, and in intravenous form. It prevents HIV by altering the genetic material of healthy T-cells. This prevents the cells from producing new virus and decreases the amount of virus in the body. Both of these antiretroviral drugs are phosphorylated in the body to their active triphosphate form by cellular kinases and selectively inhibit nucleoside reverse transcriptase (RNA dependent DNA polymerase that HIV needs to make more copies of itself). Combination of Lamivudine and Zidovudine, as a single tablet at recommended dose of 150 mg and 300 mg respectively is superior to monotherapy in treatment of HIV infections because, monotherapy with any single antiretroviral agent is no longer considered an acceptable option in the treatment of HIV infection. This combination is also used to treat health-care workers or other individuals exposed to HIV infection after accidental contact with HIV-contaminated blood, tissues, or other body fluids[1-3].

Lamivudine is reported to be estimated by HPLC method for chiral separation[4]. Two similar HPLC methods specify estimation of Zidovudine[5, 6]. Only one RP-HPLC method is reported for simultaneous determination in tablet formulation[7]. The aim of present study is development of simple, accurate and economical spectrophotometric method for simultaneous assay of both drugs in pharmaceutical preparations.

Experimental

A Thermo Spectronic Genesys-2 spilt beam, dual detector UV/Vis recording spectrophotometer with spectral band width of 2 nm was employed for all spectroscopic measurements using a pair of 10 mm matched quartz cells. Lamivudine and Zidovudine were

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obtained as gift samples from M/s Ranbaxy Laboratories Ltd, Dewas (M.P). All glass double distilled water was used in the present investigation.

Standard stock solutions of both drugs 200µg/ml each was prepared separately in distilled water and suitably diluted to different concentrations. Linearity was observed in the concentration range 0 – 50µg/ml for both at respective absorbance maximas i.e. 268 nm for Lamivudine and 267 nm for Zidovudine.

Simple overlain spectra (fig-1) reveals both drug have very similar spectra and even the absorbance maxima very close to each other, separated by 1 nm only.

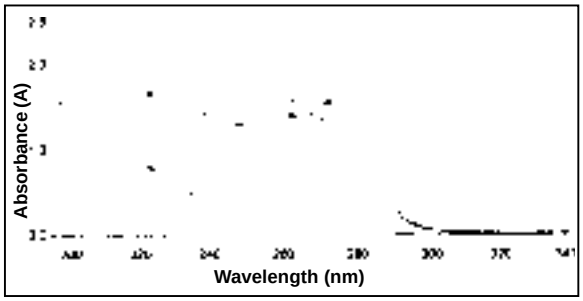

Fig. 1 : Overlain Spectra of Lamivudine (▲) and Zidovudine (♦)

Hence utilizing various approaches, direct spectrophotometric determinations of both drugs in multicomponent dosage forms is too much complicated by spectral overlapping in the whole wavelength region and even virtually makes it impossible in this case. In an approach to simplify the situation the spectra of both drugs were derivatised to first order between 200 nm and 340 nm with $\delta\lambda$ of 3 nm using a scan speed of 1800 nm/min. The overlain first derivative spectra revels that Lamivudine has zero amplitude ($DA=dA/d\lambda=0$) at 249.5 nm where, Zidovudine show substantial amplitude. Similarly Zidovudine has zero amplitude ($DA=dA/d\lambda=0$) at 235.4 nm, where Lamivudine show considerable amplitude. Hence Lamivudine and Zidovudine can be estimated at 235.4 and 249.5 nm respectively in a mixture without interference from other in their first derivative spectra.

The first derivative amplitude at selected wavelengths i.e.235.4 nm and 249.5 nm ($DA_{235.4}$, $DA_{249.5}$) of different dilutions of Lamivudine and Zidovudine in the range of 0-50 µg/ml were recorded. Working calibration curve were plotted and evaluated by least squares method.

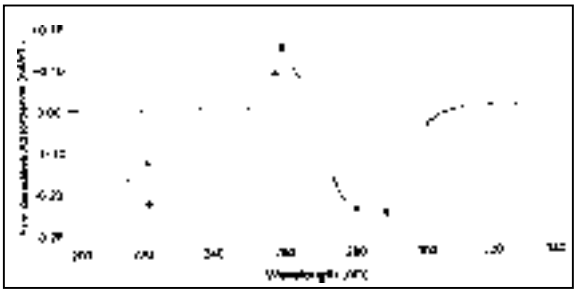

Fig. 2 : Overlain First Derivative Spectra of Lamivudine (▲) and Zidovudine (♦)

The following linear regression equations were obtained and utilized for direct estimation of both drugs in multicomponent samples.

$C_L = -714.29 \times DA_{235.4} - 0.00015$ …(1)
(r=0.9999)

$C_Z = 333.15 \times DA_{249.5} - 0.8194$ …(2)
(r=0.9985)

Where, C_L and C_z are concentration of Lamivudine and Zidovudine respectively.

Seven mixed standards of both drugs in different ratio were prepared and the first derivative amplitudes at selected wavelengths were recorded. By employing equations (1) and (2) the concentration of both drugs were worked out. The results of analysis were validated statistically and are shown in table 1.

Table 1: *Analysis of Authentic Samples (n=7)*

Analyte	C.I.	SD	%SE	't'
Lamivudine	98.911 ± 1.4359	1.938	0.732	0.1877
Zidovudine	99.223 ± 1.1015	1.486	0.562	0.2161

SD: Standard deviation, %SE: Percent standard error, C.I. (Confidence Interval within which true value may be found at 95% confidence level) = $R \pm ts/\sqrt{n}$, where R is mean percent result of analysis of authentic samples, t: theoretical 't' values at 95% confidence level for n-1 degrees of freedom t(0.05,6)=2.447.

Commercial tablets (each tablet containing Lamivudine 150 mg and Zidovudine 300 mg) procured from local market were used for analysis. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder sample equivalent to 100 mg of Zidovudine was transferred to a 100 ml volumetric flask. The powder was dissolved in about 75 ml of water with shaking. The volume was made up to mark and

filtered through Whatman Filter paper no 41. The filtrate was suitably diluted to get a final concentration of 12.5 μg/ml of Lamivudine and 300 μg/ml of Zidovudine. The first derivative amplitudes at specified wavelengths were recorded and the concentration of both drugs were worked out utilizing equations (1) and (2). The results of analysis are shown in Table 2.

Table 2: *Analysis of Commercial Tablets (n=4)*

Analyte	Label Claim (mg/tab)	Amount Found (mg/tab)	C.I.	SD
Lamivudine	150	147.856	98.57± 2.799	2.857
Zidovudine	300	306.991	102.33± 0.653	0.666

Here, R is mean percent of label claim of commercial sample; t (0.05,3)=3.182

To study the recovery of both drugs, preanalyzed samples were taken to which different quantities of pure drugs (reference standards) were added within the analytical concentration range limitations in proposed method. The added quantities of individual drugs were estimated by proposed method.

Results And Discussions

Derivative spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences in assay of two component mixtures using the zero crossing technique[8]. The technique resolves the overlapped interference by smoothing peaks and loss of background signals and so increases sensitivity of detection[9]. The component being determined should make a reasonable contribution to the total derivative amplitude of the mixture at selected wavelengths[8]. Optimum resolution of complex interferences is achieved through first order derivatisation of normal spectra with $\delta\lambda$ of 3 nm. Zidovudine also show another zero crossing point in vicinity of 267 nm where, Lamivudine could also be estimated, but due to non-linearity of Lamivudine, with a steep slope[8] of Zidovudine (shifting of zero crossing) and a preliminary investigation rejected this as sampling wavelength. The proper selection of wavelengths eliminates the spectral interference.

The modalities adopted in experimentation were successfully validated as per standard analytical procedures. Prior to analysis of commercial formulations both methods were validated by preliminary analysis of authentic laboratory samples (mixed standards) containing both drugs in different ratio. The results of analysis of authentic samples were compared with theoretical value of 100 percent by means of student's 't' test at a 95 percent confidence level. Recovery studies were also carried out and found satisfactory in the range of 99% to 102% with standard deviation values well below 2. Both analysis of authentic samples and recovery study showed there was no interference from common adjuvants used in the formulation indicating accuracy and reliability of both methods. The results of analysis of commercial formulations are found to be satisfactory with standard deviation values within acceptable limits (table 2). The proposed method has been found to be simple, convenient, and suitable for routine analysis in laboratory and shows adherence to sensitivity, accuracy and precision.

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