



# Spectra resolution for simultaneous spectrophotometric determination of lamivudine and zidovudine components in pharmaceutical formulation of human immunodeficiency virus drug based on using continuous wavelet transform and derivative transform techniques



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## ABSTRACT

In the present paper, two spectrophotometric methods based on signal processing are proposed for the simultaneous determination of two components of an anti-HIV drug called lamivudine (LMV) and zidovudine (ZDV). The proposed methods are applied to synthetic binary mixtures and commercial pharmaceutical tablets without the need for any chemical separation procedures. The developed methods are based on the application of Continuous Wavelet Transform (CWT) and Derivative Spectrophotometry (DS) combined with the zero cross point technique. The Daubechies (db5) wavelet family (242 nm) and Dmey wavelet family (236 nm) were found to give the best results under optimum conditions for simultaneous analysis of lamivudine and zidovudine, respectively. In addition, the first derivative absorption spectra were selected for the determination of lamivudine and zidovudine at 266 nm and 248 nm, respectively. Assaying various synthetic mixtures of the components validated the presented methods. Mean recovery values were found to be between 100.31% and 100.2% for CWT and 99.42% and 97.37% for DS, respectively for determination of LMV and ZDV. The results obtained from analyzing the real samples by the proposed methods were compared to the HPLC reference method. One-way ANOVA test at 95% confidence level was applied to the results. The statistical data from comparing the proposed methods with the reference method showed no significant differences.

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## 1. Introduction

In recent years, the number of patients diagnosed with Human Immunodeficiency Virus (HIV) and consequently Acquired Immunodeficiency Syndrome (AIDS) has increased dramatically. HIV is a type of retrovirus. Antiretroviral drugs treat infections caused by retroviruses. The purpose of antiretroviral treatment is to sustain HIV at a low level in the body [1]. A group of antiretroviral drugs is Nucleoside Reverse Transcriptase Inhibitors (NRTIs). These inhibitors interfere in the HIV reverse transcription process (from RNA to DNA) by incorporating into the cellular target of HIV. This ultimately prevents viral growth. These target cells are known as CD4 receptors. In order to incorporate NRTIs to the CD4 receptors, it is necessary to activate the NRTIs. Adding three phosphate groups to form 5'-triphosphate nucleotide activates the NRTIs. Cellular kinase enzymes carry out this phosphorylation step [2,3].

Since single drug treatment quickly became ineffective due to the development of HIV resistant strains. A new technique is to combine two to four antiretroviral drugs. This is the Highly Active Antiretroviral Therapy (HAART) technique [1]. One such product is Co-Biovir<sup>®</sup>, which is a combination of lamivudine and zidovudine in a single tablet. Both lamivudine and zidovudine belong to the group of NRTIs.

The chemical name of lamivudine is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Similarly the chemical name of zidovudine is 1-[(2R, 4S, 5S)-4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-1, 2, 3, 4-tetrahydropyrimidine-2, 4-dione [4,5]. Fig. 1 shows the chemical structures of these two drugs.

Surveys conducted on published literature demonstrate that several analytical methods exist for quantitative determination of these two drugs in various biological tissues and pharmaceutical samples.

Analytical methods reported for determination of LMV or ZDV include normal phase HPTLC [6], LC-MS/MS [7,8] and Reverse Phase Liquid Chromatography (RPLC-UV) [3,9–11].

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In addition, some reports involved simultaneous determination of binary mixture of lamivudine and zidovudine. These analytical methods included reverse phase HPLC–UV [12–14], HPLC with tandem mass spectrometry [1,2,15–17], normal phase HPTLC [5,18] and micellar electrokinetic chromatography [19]. These methods have several disadvantages such as using highly pure solvents that are commonly environmental contaminants, time consuming and require rather expensive and complicated analytical equipments.

Recently, chemometrics methods are rapidly developed and widely applied in different fields of chemistry, especially analytical chemistry. One of the techniques of chemometrics is the multivariate calibration method [20]. Some studies have used these methods for determination of lamivudine together with other drugs [21]. Another technique used for the assay of LMV and ZDV is the Derivative Spectrophotometry (DS) method [4,22].

Nowadays the Wavelet Transform (WT) method has many applications in various fields [23–31], especially in pharmaceutical sciences [32–41].

Coupling wavelet transform with the zero cross point technique increases spectra resolution and reduces interference due to spectrum overlapping. Therefore, we can perform simultaneous quantitative multi-component mixture measurement without requiring separation of components.

The aim of this study is to propose two spectrophotometric signal-processing methods of continuous wavelet transform and

derivative spectrophotometry for simultaneous determination of binary mixtures of lamivudine and zidovudine in the commercial pharmaceutical products without any separation steps. To find the best point for assay, these methods were combined with the zero cross point technique. The proposed methods were validated by synthesizing various mixtures. In addition, results obtained were compared to the reference method (HPLC) by one-way ANOVA test. The results achieved show a good agreement.

## 2. Experimental section

### 2.1. Apparatus and software

#### 2.1.1. Spectrophotometry

Spectrophotometric analysis was carried out with a Cary 100 Bio UV–visible double beam spectrophotometer (Agilent Technologies) equipped with 1.0 cm quartz cells. The absorption spectra of solutions were recorded over the range of 200–300 nm at 1 nm intervals with respect to a blank of deionised water.

The spectrophotometric measurements were carried out at room temperature (mean of about 20 °C) and all solutions were prepared on the same day before that of the analysis.

A model pHs-3C pH meter was calibrated by using buffer solutions and then used for pH measurements. The pH of the solutions was surveyed in the range of pH 3–9.

All data treatment calculations were performed by transferring the spectral data to the Wavelet Toolbox of MATLAB 7.12 software and Microsoft Excel 2007 program.

#### 2.1.2. High performance liquid chromatography

The HPLC system (Agilent 1260 infinity) used was equipped with UV 1260 infinity diode array detector set to 270 nm. Separations were carried out using a reverse-phase C<sub>18</sub> column (4.6 mm × 150 mm, 5 μm particle sizes). The mobile phase was prepared with a mixture of acetonitrile and potassium phosphate buffer (20:80, v/v). Samples were eluted with a flow rate of 1 mL min<sup>-1</sup>. The mobile phase was adjusted to pH 7 with NaOH 2.5 N [42].

### 2.2. Chemicals and reagents

Pure lamivudine and zidovudine drugs and its pharmaceutical dosage form (named Co-Biovir<sup>®</sup>) containing 150 mg of LMV and 300 mg of ZDV were donated by Bakhtar Bioshimi Company.

Potassium phosphate buffer and acetonitrile were HPLC grade and obtained from Merck (Darmstadt, Germany). Sulfuric acid and NaOH were purchased from Merck (Darmstadt, Germany).

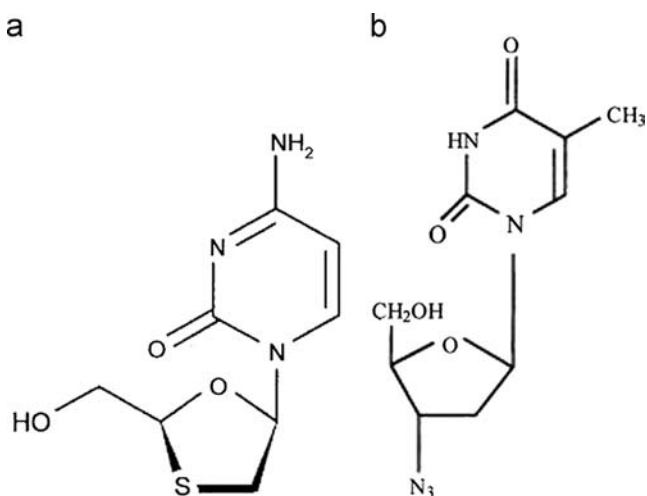


Fig. 1. Chemical structures of (a) lamivudine and (b) zidovudine.

Table 1

Statistical results of calibration standard solutions graphs obtained by the proposed and reference methods.

Parameter	CWT		FDS		HPLC	
	LMV	ZDV	LMV	ZDV	LMV	ZDV
Wavelength (nm)	242	236	266	248	–	–
Linear range (μg mL <sup>-1</sup> )	5–50	5–50	5–50	5–50	5–50	5–50
Slope (a)	–0.0584	–0.0465	0.0006	0.0014	45.635	40.148
Intercept (b)	0.0041	0.0053	0.0003	–5E–06	–19.224	–29.485
Regression coefficient (R <sup>2</sup> )	0.9997	1	0.9989	0.9998	0.9997	0.9995
Correlation coefficient (r)	–0.9998	–0.99998	0.9995	0.9999	0.9998	0.9997
LOD (μg mL <sup>-1</sup> )	0.84	0.32	1.71	0.68	0.9	1.23
LOQ (μg mL <sup>-1</sup> )	2.79	1.07	5.69	2.28	3.01	4.09

a, slope of the regression function; b, intercept of the regression function; R<sup>2</sup>, Regression coefficient; r, correlation coefficient of the regression function; LOD, limit of detection; LOQ, limit of quantification.

**Table 2**  
Results from application of CWT and FDS on laboratory prepared mixtures.

Added ( $\mu\text{g mL}^{-1}$ )		CWT						FDS					
		Founded <sup>a</sup> ( $\mu\text{g mL}^{-1}$ )		Standard error (%)		Recovery (%)		Founded ( $\mu\text{g mL}^{-1}$ )		Standard error (%)		Recovery (%)	
LMV	ZDV	LMV $\lambda=242$ (nm)	ZDV $\lambda=236$ (nm)	LMV	ZDV	LMV	ZDV	LMV $\lambda=266$ (nm)	ZDV $\lambda=248$ (nm)	LMV	ZDV	LMV	ZDV
15	15	15.05	14.95	0.31	0.3	100.31	99.7	15.03	14.46	0.2	3.63	100.2	96.37
10	20	10.06	19.88	0.59	0.59	100.59	99.41	9.86	19.27	1.37	3.65	98.63	96.35
5	15	4.97	15.02	0.5	0.13	99.5	100.13	4.94	14.63	1.23	2.47	98.767	97.53
10	5	10.04	5.01	0.42	0.26	100.42	100.26	9.84	4.87	1.57	2.63	98.43	97.37
30	10	30.22	10.15	0.72	1.5	100.72	101.5	30.32	9.92	1.06	0.8	101.06	99.2
Mean		–	–	0.51	0.56	100.31	100.20	–	–	1.084	2.63	99.42	97.37
R.S.D. <sup>b</sup>		–	–	–	–	0.48	0.80	–	–	–	–	1.16	1.19
R.S.D. <sup>c</sup>		–	–	–	–	0.69	1.03	–	–	–	–	1.67	1.55

R.S.D., relative standard deviation.

<sup>a</sup> Mean value of the three determinations.

<sup>b</sup> Intra day.

<sup>c</sup> Inter day.

The water used in all studies was deionised water supplied by a Milli-Q deionization system.

### 2.3. Standard solutions

Stock solutions of lamivudine and zidovudine ( $100 \mu\text{g mL}^{-1}$ ) were prepared separately by dissolving 50 mg for each compound in 500 mL deionised water.

Standard calibration solutions of LMV and ZDV in the concentration range between 5 and  $50 \mu\text{g mL}^{-1}$  were individually prepared from the aforementioned stock solutions (Table 1). This concentration range obeys the Beer Lambert law and amplitudes of the obtained peaks are suitable for simultaneous determination of the two components. The concentrations used for each analyte in the linear range of  $5\text{--}50 \mu\text{g mL}^{-1}$  consisted of 10 levels (with  $5 \mu\text{g mL}^{-1}$  intervals).

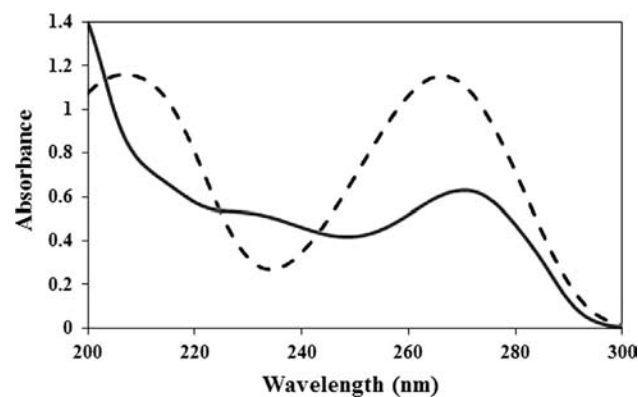
In order to validate the proposed methods, the standard synthetic mixtures containing two compounds were also prepared by using the same stock solutions. These synthetic mixtures are shown in Table 2.

### 2.4. Analysis of commercial tablets

In order to assay the pharmaceutical, ten tablets were weighed individually to obtain representative average weights. The tablets were finely powdered and mixed. A mass corresponding to one tablet was accurately weighed and transferred to a  $250 \text{ mL}^{-1}$  beaker and dissolved in  $200 \text{ mL}^{-1}$  deionized water. The solution was placed on a magnetic stirrer for 30 min and filtered through a Whatman no. 41 filter paper. After filtration, the obtained clear solution was adjusted to the volume of 1000 mL with the same solvent. This solution was further diluted to get the suitable concentration for the UV measurements [20].

## 3. Wavelet transform method

Wavelet transform analysis, transforms a signal into another form. The new signal provides better information in a more useful form. This signal transformation is the wavelet transform. The wavelet transform, converts signals from the time domain into the time–frequency domain and it includes the continuous and discrete parts. Wavelet transform functions perform the wavelet transform.



**Fig. 2.** The absorption spectra of lamivudine  $15 \mu\text{g mL}^{-1}$  (—) and zidovudine  $30 \mu\text{g mL}^{-1}$  (- - -).

The mother wavelet is the original wavelet transform function. Eq. (1) defines this function as

$$\Psi_{b,a}(t) = \frac{1}{\sqrt{a}} \psi\left(\frac{t-b}{a}\right) \begin{cases} a, b \in R \\ a \neq 0 \end{cases} \quad (1)$$

where  $a$  is the scale or the dilation parameter and it is used to change the scale,  $b$  is the location parameter which is used to move the wavelet along the time axis and  $R$  is the domain of real numbers.

Eq. (2) defines the continuous wavelet transform function as:

$$CWT(b, a) = C(b, a) = \int_{-\infty}^{\infty} x(t) \frac{1}{\sqrt{a}} \psi^*\left(\frac{t-b}{a}\right) dt \quad (2)$$

The \* indicates that the complex conjugate is used in case of a complex wavelet. The signal energy is normalized at every scale by dividing the wavelet coefficients by  $1/\sqrt{a}$ . This ensures that the wavelets have the same energy at every scale [43].

There are different types of mother wavelets. In this study, we used the Daubechies and Dmey wavelet families.

## 4. Result and discussion

### 4.1. UV spectra and proposed methods

Fig. 2 illustrates the original absorption spectra of pure lamivudine and zidovudine. This spectrum shows that both drugs

strongly overlap with each other in the wavelength range of 200–300 nm.

The pH changes of the samples had no effect in separation or reduction of overlapping spectra. Due to their mutual interference, simultaneous determination of the binary mixture of LMV and ZDV is not possible by using classical spectrophotometric procedures.

In order to overcome this problem, we proposed the continuous wavelet transform and the derivative spectrophotometry methods in combination with the zero cross-point technique.

#### 4.2. Continuous wavelet transform

To run the continuous wavelet method for simultaneous determination of the two drugs, first the data of the standard calibration solutions obtained from the UV spectrophotometer in an EXCEL file were transferred into the wavelet environment.

Afterwards all of the wavelet families processed the data and different scale parameters were changed to get the optimal scale values. In the following step, the CWT spectrum of each component was obtained by plotting  $C_{a,b}$  coefficients against wavelengths between 200 and 300 nm.

Subsequently, the CWT spectra of two components were situated in the presence of each other and the zero cross point technique was applied on them.

Finally, the Daubechies wavelet family of five order (db5) with scaling factor ( $a$ )=54 in the wavelength of 242 nm (corresponding to zero cross-point of zidovudine) and Dmey wavelet family using  $a$ =28 in the wavelength of 236 nm (corresponding to zero cross-point of lamivudine) were found to give the best results under optimum conditions for simultaneous analysis of lamivudine and zidovudine, respectively.

These wavelet families produced the highest  $R^2$  and slope values in the linear regression equations while producing the least relative error percentage in all of the solutions during determination of the two components.

Fig. 3a and b depict CWT graphs of the standard calibration solutions of LMV and ZDV respectively in the concentration range of 5–50  $\mu\text{g mL}^{-1}$ . These graphs were obtained by measuring values of db5 ( $a$ =54) and Dmey ( $a$ =28) in the corresponding wavelengths for LMV and ZDV, respectively.

Linear regression graphs of each component were constructed by plotting the amount of the calibration solutions' CWT signal amplitude in the corresponding wavelengths versus the related concentrations. These graphs created linear regression functions that were used for prediction of unknown concentrations of two components in binary mixtures. Table 1 summarizes statistical parameters obtained from linear regression equations.

#### 4.3. Derivative transform

The second proposed method in this study was based on derivative UV–spectrophotometry combined with zero-crossing point technique.

Different spectrophotometric derivative orders were evaluated to find the optimal processed signal for obtaining desirable calibration graphs and reliable determination of the investigated drugs. Finally, the first derivative ( $D_1$ ) of the absorption spectra was selected as the best order for determination of the two components, thus lamivudine was determined at 266 nm where the  $dA/d\lambda$  amplitude intensity of zidovudine is zero or near zero and similarly zidovudine was determined at 248 nm (corresponding to zero cross-point of lamivudine).

Fig. 3c shows calibration graphs of derivative spectrophotometry of the two drugs. Measuring the  $dA/d\lambda$  amplitude values at 266 nm and 248 nm and plotting them against corresponding standard

solutions concentrations produced the linear regression graphs and their functions for LMV and ZDV, respectively. Table 1 shows the statistical results obtained from linear regression graphs.

#### 4.4. Reference method

The HPLC method was used as the reference method. Samples were eluted with a flow rate of 1  $\text{mL min}^{-1}$  using a mobile phase consisting of acetonitrile and potassium phosphate buffer (20:80, v/v) at the wavelength of 270 nm.

As shown in Fig. 4 the retention times of LMV and ZDV were found to be 2.4 and 5.3 min respectively.

The calibration curves for two compounds were obtained by plotting peak areas against corresponding concentrations in the range of 5–50  $\mu\text{g mL}^{-1}$ . Table 1 presents the linear regression parameters of this method.

#### 4.5. Validation of proposed methods

The proposed methods were validated by synthesizing various mixtures containing lamivudine and zidovudine. The statistical results achieved are presented in Table 2.

The high values of correlation coefficients ( $r$  bigger than 0.999) indicated the good linearity of all measured values in the linear concentration range of 5–50  $\mu\text{g mL}^{-1}$  for both of the drugs.

The excellent recoveries obtained were 99.5–100.7% for LMV and 99.4–101.5% for ZDV, which suggest the high accuracy of the proposed methods.

In addition, the Relative Standard Deviation (R.S.D.) for all determinations was less than 1.7% that demonstrated the high precision of the methods.

The Limit of Detection (LOD) is the analyte concentration producing a signal equal to the blank signal,  $y_B$ , plus three standard deviations of the blank signal,  $s_B$  ( $y_B + 3s_B$ ). Similarly, the Limit of quantification (LOQ) is the analyte concentration giving a signal equal to the blank signal,  $y_B$ , plus ten standard deviations of the blank signal,  $s_B$  ( $y_B + 10s_B$ ) [44]. So by using the obtained values and regression equations, LOD and LOQ for lamivudine were found to be 0.84 and 2.79  $\mu\text{g mL}^{-1}$ , respectively while for zidovudine the corresponding values were 0.32 and 1.1  $\mu\text{g mL}^{-1}$ , respectively.

#### 4.6. Application to a commercial formulation of tablet

The proposed methods were applied to simultaneous quantitative determination of lamivudine and zidovudine in Co-Biovir<sup>®</sup> tablets. The results obtained by this analysis are listed in Table 3.

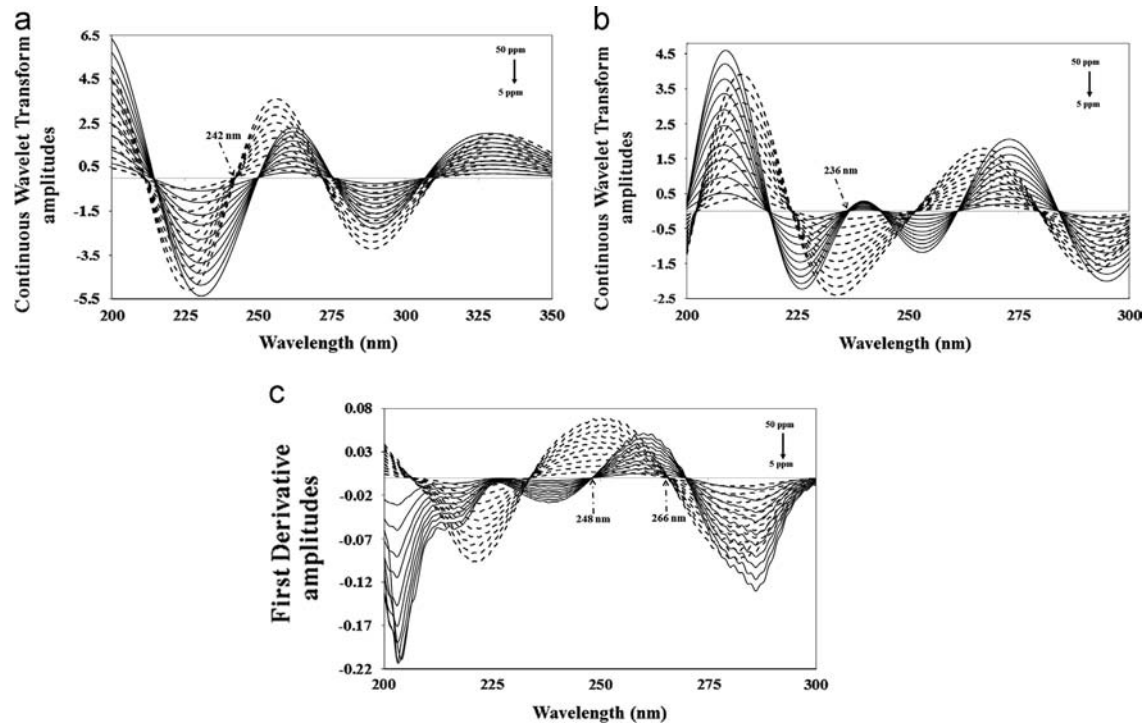
One-way ANOVA test was applied in order to survey the presence or absence of a significant difference between the results of both proposed methods and results achieved by the reference method. Table 4 summarizes the data obtained by this comparison.

Since the calculated  $F$ -values (3.01 for LMV and 3.64 for ZDV) were less than the critical  $F$ -values (5.14), it was concluded that at 95% confidence level there were no significant errors found in determination of the drugs by the proposed methods.

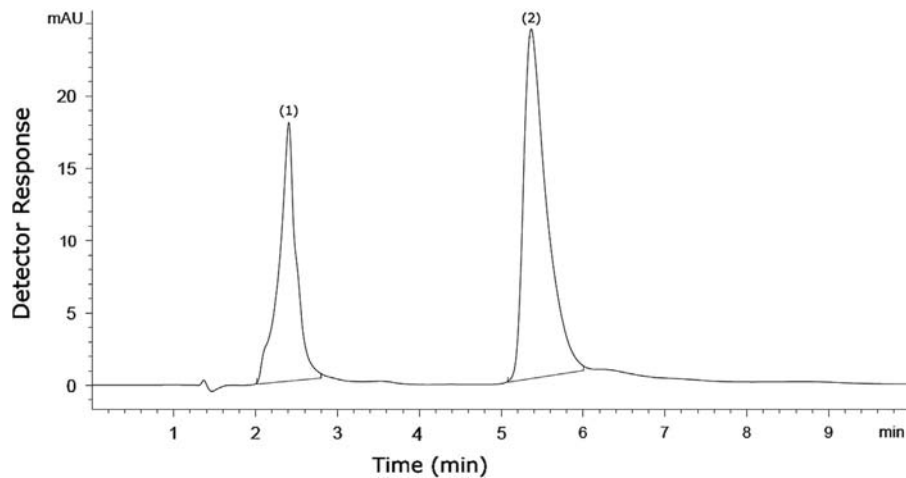
Thus, the proposed methods are sufficiently accurate and precise in order to be applied to pharmaceutical dosage form.

## 5. Conclusion

Since the absorption spectra of binary mixture of lamivudine and zidovudine strongly overlap with each other, we used the Continuous Wavelet Transform (CWT) and Derivative Spectrophotometry (DS) methods combined with the zero cross point



**Fig. 3.** Spectra of (a) CWT-db5, (b) CWT-Dmey and (c) first derivative for different concentrations of (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50  $\mu\text{g mL}^{-1}$ ) lamivudine (—) and zidovudine (- - -).



**Fig. 4.** Chromatogram obtained from commercial formulation of tablet containing: (1) 150 mg of lamivudine, and (2) 300 mg of zidovudine.

**Table 3**

Results obtained by the proposed and reference methods to the commercial tablet of Co-Biovir<sup>®</sup>.

Method	CWT (Intra day)		CWT (Inter day)		FDS (Intra day)		FDS (Inter day)		HPLC	
	LMV $\lambda=242$ (nm)	ZDV $\lambda=236$ (nm)	LMV $\lambda=242$ (nm)	ZDV $\lambda=236$ (nm)	LMV $\lambda=266$ (nm)	ZDV $\lambda=248$ (nm)	LMV $\lambda=266$ (nm)	ZDV $\lambda=248$ (nm)	LMV	ZDV
<b>Label Claim (mg)</b>	150	300	150	300	150	300	150	300	150	300
<b>Amount Found (mg)<sup>a</sup></b>	149.26	299.35	148.78	299.18	148.89	297.2	152.07	297.39	148.34	297.25
<b>S.D.</b>	0.2574	0.2587	0.9821	0.4082	0.3056	0.4259	2.173	1.1581	1.382	1.5226
<b>R.S.D. (%)</b>	0.1724	0.08643	0.6601	0.1364	0.2053	0.1433	1.4289	0.3894	0.9316	0.5122
<b>S.E. (%)</b>	0.49	0.22	0.81	0.27	0.74	0.93	1.38	0.87	1.55	0.92
<b>Recovery (%)</b>	99.51	99.78	99.19	99.73	99.26	99.07	101.38	99.13	98.45	99.08

<sup>a</sup> Mean value of the three determinations.

**Table 4**  
The ANOVA test results by applying three methods to the real samples.

Source of variation	ss	df*	MS	Calculated F-value	Critical F-value
<b>Between groups</b>					
Lamivudine	4.151957	2	2.075979	3.009394	5.143253
Zidovudine	9.03176	2	4.51588	3.636057	5.143253
<b>Within groups</b>					
Lamivudine	4.138997	6	0.689833		
Zidovudine	7.451831	6	1.241972		
<b>Total</b>					
Lamivudine	8.290954	8			
Zidovudine	16.48359	8			

ss, sum of squares; df, degree of freedom; MS, mean squares.

\* Degree of freedom for between groups:  $h - 1$ ; Within Groups:  $h(n - 1)$ ; Total:  $hn - 1$ ;  $h$ , number of methods;  $n$ , number of samples of each method.

technique. These two methods lead to spectra resolution, therefore, simultaneous quantitative determination of the mentioned components in synthetic mixtures and commercial samples can be done.

Comparing the results achieved from the proposed methods and the HPLC reference method, show no significant differences between them and thus, the results are satisfactory. Therefore, it is indicated that the two methods developed are sufficiently accurate and precise. However, the results of CWT were better than DS. The principal advantage of CWT is the high amplitude of wavelet transform graphs that leads to increase in the Signal to Noise ratio ( $S/N$ ) and it improves sensitivity. In addition, the proposed procedures are simple, fast, economic and do not require initial pretreatment steps. Consequently, these methods are appropriate for quality control laboratories.

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