Continuous Wavelet Transforms for the Simultaneous Quantitative Analysis and Dissolution Testing of Lamivudine–Zidovudine Tablets

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Dissolution testing has a very vital importance for a quality control test and prediction of the *in vivo* behavior of the oral dosage formulation. This requires the use of a powerful analytical method to get reliable, accurate and precise results for the dissolution experiments. In this context, new signal processing approaches, continuous wavelet transforms (CWTs) were improved for the simultaneous quantitative estimation and dissolution testing of lamivudine (LAM) and zidovudine (ZID) in a tablet dosage form. The CWT approaches are based on the application of the continuous wavelet functions to the absorption spectra-data vectors of LAM and ZID in the wavelet domain. After applying many wavelet functions, the families consisting of Mexican hat wavelet with the scaling factor a=256, Symlets wavelet with the scaling factor a=512 and the order of 5 and Daubechies wavelet at the scale factor a=450 and the order of 10 were found to be suitable for the quantitative determination of the mentioned drugs. These wavelet applications were named as mexh-CWT, sym5-CWT and db10-CWT methods. Calibration graphs for LAM and ZID in the working range of 2.0–50.0 μg/mL and 2.0–60.0 μg/mL were obtained measuring the mexh-CWT, sym5-CWT and db10-CWT amplitudes at the wavelength points corresponding to zero crossing points. The validity and applicability of the improved mexh-CWT, sym5-CWT and db10-CWT approaches was carried out by the analysis of the synthetic mixtures containing the analyzed drugs. Simultaneous determination of LAM and ZID in tablets was accomplished by the proposed CWT methods and their dissolution profiles were graphically explored.

Key words continuous wavelet transform; determination; dissolution testing; anti-human immunodeficiency virus (HIV) drug; tablet

Lamivudine (LAM), chemically named (2*R*,*cis*)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1*H*)-pyrimidin-2-one or (−)-2′,3′-dideoxy, 3′-thiacytidine (3TC) is the (−)-enantiomer of a dideoxy analogue of cytidine. LAM has been used for the treatment of infections with the human immunodeficiency viruses (HIVs) and chronic hepatitis B viruses. LAM is phosphorylated to its active 5′-triphosphate metabolite (LAM triphosphate). The principal mode of action of LAM triphosphate is inhibition of reverse transcriptase *via* DNA chain termination after incorporation of the nucleoside analogue. LAM triphosphate is a weak inhibitor of mammalian DNA polymerases-α, -β and mitochondrial DNA polymerase-γ LAM is often given in combination whit zidovudine (ZID) in order to provide desirable therapy. ZID or azidothymidine (AZT) has been used for the treatment of HIV/AIDS infectiousness. Its chemical name is 3′-azido-3′-deoxythymidine. ZID is phosphorylated to its active 5′-triphosphate metabolite (ZID triphosphate). ZID triphosphate is inhibition of reverse transcriptase *via* DNA chain termination after incorporation of the nucleoside analogue. ZID triphosphate is a weak inhibitor of the mammalian DNA polymerase-α and mitochondrial DNA polymerase-γ and has been reported to be incorporated into the DNA of cells in culture. In HIV-1 infected MT-4 cells, LAM in combination with ZID had synergistic antiretroviral activity. Synergistic activity of LAM and ZID was also shown in a variable-ratio study. LAM and ZID are in a class of medications called nucleoside reverse transcriptase inhibitors.

A review of the literature shows that various conventional analytical methods including spectrophotometry,[1–5] HPTLC,[4] HPLC,[4–13] HPLC-MS[14] and multivariate calibration method[15] were reported for the analysis of LAM and ZID or their combination with other drugs in tablets, human serum and in drug dissolution studies. However, there is no continuous wavelet transform (CWT) method reported for the simultaneous quantitative estimation of both drugs.

As it has been known HPLC is a standard and comparison method for the analysis of combined pharmaceutical preparations and can be coupled with mass spectrometry such as LC-MS and LC-MS-MS to obtain a new and advanced technique called hyphenated instrumentation using two or more devices simultaneously for quantitative measurements. In some cases, HPLC may not give a suitable chromatographic separation due to similarity of the physical and chemical properties of analyzed compounds. In this case, a qualitative and quantitative analysis is unable to perform by using unresolved peaks in a chromatogram. This unusual case requires additional experiment, tedious procedures and use of expensive chemicals or solvents to get optimal chromatographic separation conditions. As a result, HPLC technique using very expensive apparatus components brings high cost and time consumption to reach desirable analysis results.

UV-VIS spectrophotometry, especially, derivative spectrophotometry has been extensively used for resolving complex mixture, particularly two-component mixture without preliminary separation procedure because of its simplicity and providing fast analysis with low cost. In spite of the mentioned advantages, derivative spectrophotometry may not always provide us an opportunity to obtain rapidly high accuracy and reproducibility for the analysis results due to the strong overlapping spectra of drugs, interference of main spectral peak with noise band, unexpected spectral baseline error, decreas-

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ing signal intensity and worsening signal-to-noise (*S*/*N*) ratio, especially, *e.g.*, in higher derivative orders, and insufficient smoothing of the initial spectra and instrumental or methodological factors.

In recent years, popular multivariate calibration methods such as classical least squares (CLS), invers least squares (ILS), principal component regression (PCR) and partial least squares (PLS), using spectra data have been applied to the analysis of active compounds in multicomponent pharmaceutical preparations in the absence of interference of excipients. These chemometric calibration methods based on numerical calculations and data decomposition may not give us successful results for the quantitative resolution of complex samples because of the above mentioned problems of classical UV-VIS spectrophotometric approaches. In this context, wavelet transform (WT) is one way to overcome the mentioned disadvantages of the conventional separation and spectrophotometric methods for the efficient quantitative resolution of complex mixtures. WT approach can be classified into two categories; discrete wavelets transform (DWT) and CWT. For example, a chemical complex signal obtained from instrumentation recording of a spectrum is composed of baseline, noise and signal of analyzed compounds. By using the WT approach, this complex signal can be decomposed into its components with different frequencies. In this way, WT approach provides us many possibilities such as data reduction, baseline correction, and elimination of noise and resolution of overlapping peaks, since the frequencies of the mentioned components are significantly different from one another.[16] During the last decades the WT method has been used as an efficient tool to solve problems in chemistry as well as several areas of science and engineering.[17,18]

Due to the mentioned methodological properties, recently, the use of the CWT methods in combination with zero-crossing technique and ratio spectra treatment gave us reliable, sensitivity precise and accurate results for the analysis of combined pharmaceutical preparations.[19–24] CWT approaches have attracted increased interest due to the potential usage in analytical applications and various areas of science and industry. In the ref. 25, we applied only sym6-CWT (a=128) method to levodopa–benserazide combination. In this study, mexh-CWT (a=256), sym5-CWT (a=512) and db10-CWT (a=450) were subjected to the analyses and dissolution tests of LAM–ZID tablets. These are differences between the two studies. In addition, it was concluded that the analyses and the dissolution tests of the mentioned LAM–ZID tablets can be classified into three different CWT families. These wavelet families were considered to be appropriate for our study.

In this study, continuous wavelet transform approaches, mexh-CWT, sym5-CWT and db10-CWT were applied to the quantitative resolution of the strong overlapping absorption spectra for the simultaneous quantitative estimation and dissolution testing of LAM–ZID tablets without using a separation step. The validity and ability functions of the improved CWT signal processing methods were verified by analyzing synthetic samples containing the related drugs and then a good agreement for the analysis results obtained was reported.

Theoretical Aspect Today, wavelets are powerful tools as signal processing methods in chemistry as well as other fields of science; WT in the analysis of spectral signals provides many advantageous over the conventional frequency decomposition. The WT is localized in both time and frequency while Fourier transform (FT) does not give any information of the signal in the time domain. WT is alternative approaches to FT and short time STFT to overcome the resolution problem in the analysis of signals.

Wavelet is based on frequency-scale decomposition of signals. A wavelet is defined as a family of functions obtained by dilation (scaling parameter) and translation (shifting parameter).

$$\Psi^{\psi}_{a,b}(x) = \frac{1}{\sqrt{|a|}}\psi\left(\frac{x-b}{a}\right) \tag{1}$$

where a is the scaling parameter and b is the shifting parameter. The scaling parameter is an important role to change time and frequency resolution when a signal is analyzed. CWT of one-dimensional signal is defined as

$$\mathrm{CWT}^{\psi}_{x}(a,b) = \Psi^{\psi}_{x}(a,b) = \frac{1}{\sqrt{|a|}}\int x(t)\psi^{*}\left(\frac{x-b}{a}\right)\mathrm{d}t \tag{2}$$

$$\mathrm{CWT}^{\psi}_{x}(a,b) = \left\langle f(x), \psi_{a,b}\right\rangle \tag{3}$$

where the superscript * denotes the complex conjugate and $\left\langle f(x), \psi_{a,b}\right\rangle$ represents the inner product of function $f(x)$ onto the wavelet function $\psi_{a,b}(x)$.

Experimental

Instruments and Software The UV absorption spectra were recorded using Shimadzu UV-2520 double beam UV-Vis spectrophotometer connected to a computer loaded with UVProbe 2.32 software. Microsoft EXCEL and Wavelet toolbox in Matlab software were used for the spectral data treatments and statistical calculations. The *in vitro* dissolution studies were performed on Distek Evolution 6100 Dissolution System (North Brunswick, NI, U.S.A.).

Pharmaceutical Tablet Dosage Form A commercial pharmaceutical preparation, COMBIVIR® film-coated tablet (produced by Glaxo Smith Kline) containing 150 mg of LAM, 300 mg of ZID, and the inactive ingredients colloidal silicon dioxide, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide was analyzed by the proposed mexh-CWT, sym5-CWT and db10-CWT methods.

Dissolution Testing of Tablets Dissolution rate of LAM and ZID from tablets was simultaneously determined using USP Apparatus II (paddle method) in 900 mL of pH 1.2 buffer at 50 rpm and at 37±0.5°C. During the dissolution testing, the collection of the samples was made by using an injector with membrane filter (0.20 μm). The dissolution medium consisting of pH 1.2 buffer containing % 0.02 Polysorbate 20 (Tween 20) (w/v) was prepared by using 7 mL of hydrochloric acid (37.4%), 2 g of sodium chloride in 1 L of distilled water. After that the same volume of the fresh dissolution medium was added back into system. In the sampling procedure, 3 mL of sample was taken manually at 2, 5, 10, 15, 20, 30, 45 and 60 min. The dissolution testing procedure was performed on six parallel experiments. The final collected dissolution samples were diluted by using the dissolution medium into calibration concentration range of LAM and ZID drugs. The proposed mexh-CWT, sym5-CWT and db10-CWT approaches were applied to the UV spectra of the dissolution samples for the quantification and dissolution testing of the subjected

drugs.

Standard Calibration Solutions The standard stock solutions of LAM and ZID were prepared by dissolving 10 mg of each drug in 100 mL of the pH 1.2 buffer solutions. The individual calibration solutions of LAM and ZID in the linear concentration rages of 2.0–50.0 μg/mL and 2.0–60.0 μg/mL were prepared from the stock solutions, respectively. For the method applicability and validity testing of the improved CWT methods, an independent set of mixtures consisting of LAM and ZID in the mentioned working concentration ranges were prepared using the same stock solutions. The buffer solution (pH 1.2) was used during the preparation of all the samples. All the standard solutions were prepared daily and all measurements were made at room temperature.

Preparation of Tablet Sample Twenty tablets containing LAM and ZID were weighed and powdered into uniform size in a mortar. An accurately weighed portion from this powder equivalent to one tablet was transferred to a 100 mL volumetric flask containing 20 mL of the buffer solution (pH= 1.2). The content of the flask was sonicated for about 20 min for complete solubility of the drug and the volume was made up to 100 mL with the same solvent. Then the tablet sample solution was filtered through 0.45 μm membrane filter. The resulting sample solution was diluted by using the dissolution medium into working concentration range of the analyzed drugs. The UV absorption spectra of the tablet sample were recorded and processed by the improved CWT methods for the simultaneous quantitative estimation of LAM and ZID in the tablet dosage form. The analysis of LAM–ZID tablets were repeated ten times.

Results and Discussion

Signal Processing Tools and Method Development The most important problem in the application of the CWT signal processing tools for resolving the overleaping spectra of drugs in combined pharmaceuticals is to identify the optimal wavelet family at a suitable scaling factor providing an exact and well specified zero crossing point for the construction of a good calibration graph. For this aim, some applications of several continuous wavelet families at different scaling factors for the UV spectra data vectors of LAM and ZID drugs, and their samples were tested for finding optimal wavelet family. According to these pre-tests, Mexican hat CWT with the scaling factor a=256 (mexh-CWT), Symlets CWT with the scaling factor a=512 and the order of 5 (sym5-CWT) and Daubechies CWT with the scaling factor a=450 and the order of 10 (db10-CWT) were considered to be more appropriate families to obtain reliable, accurate and reproducible results for the analysis and dissolution test procedures of LAM and ZID. Under optimized signal processing settings, the improved mexh-CWT, sym5-CWT and db10-CWT signal processing tools were applied to the simultaneous quantitative estimation and dissolution testing of LAM and ZID in commercial tablets.

Application of CWT Signal Processing Methods The UV absorption spectra of the calibration solutions of LAM and ZID in the working range of 2.0–50.0 μg/mL and 2.0–60.0 μg/mL were recorded between 200–300 nm with a step of 0.05 nm, respectively as displayed in Fig. 1A. These UV absorption spectra consisting of 1024 data-points in the wavelength region of 210.00–312.35 nm were transferred as UV absorption spectra data vectors from EXCEL to the wavelet domain for the CWT signal processing procedure. The UV absorption spectra data vectors of LAM and ZID in the wavelet domain were processed by the mexh-CWT, sym5-CWT and db10-CWT signal processing tools and then the mexh-CWT, sym5-CWT and db10-CWT spectra were obtained by plotting the CWT coefficients ($C_{a,b}$) *versus* wavelengths in the spectral range of 210.00–312.35 nm, as illustrated as Figs. 1B–D. Similar spectral recording and CWT processing treatments were also applied to the samples consisting of the synthetic mixtures, commercial tablets and dissolution testing. The CWT spectra obtained were used for the simultaneous quantitative evaluation and dissolution testing of LAM–ZID tablets. The applications of the mexh-CWT, sym5-CWT and db10-CWT approaches for the analysis and dissolution testing procedures were explained in the following steps.

In the case of Mexican hat wavelet, mexh-CWT method (a=256) was applied to the UV spectra data vectors of pure LAM and ZID, and their samples in the wavelet domain. As a result of this transformation, the mexh-CWT spectra of LAM and ZID were obtained as indicated in Fig. 1B. As it is can be seen from Fig. 1B, the concentrations of LAM and ZID in the binary mixture were proportional to the mexh-CWT amplitudes at 250.35 and 284.50 nm (zero-crossing point for ZID) and 260.85 and 296.60 nm (zero-crossing point for LAM), respectively. In the specified wavelengths, two calibration graphs for each drug were obtained by using the least square regression analysis based on the measurements of the mexh-CWT amplitudes against the increasing concentrations of pure LAM and pure ZID. Calibration equations and their statistical results were given in Table 1. The amounts of LAM and ZID in their synthetic mixtures, commercial tablets and dissolution testing samples were determined by use of the mentioned calibration graphs.

In the same way, the UV spectra data vectors of pure LAM and ZID, and their samples in the wavelet domain were processed by the sym5-CWT approach using the scaling factor (a=512) and then the sym5-CWT spectra of the analyzed drugs and their samples were obtained. Figure 1C shows the sym5-CWT spectra of LAM and ZID in the working concentration range of 2.0–50.0 μg/mL and 2.0–60.0 μg/mL, respectively. It is clear from Fig. 1C that the concentrations of LAM and ZID were proportional to the sym5-CWT amplitudes at 287.75 nm corresponding to the zero-crossing point for ZID and 299.15 nm corresponding to the zero-crossing point for LAM), respectively. At the mentioned wavelength points, the calibration graphs for the related drugs were calculated by using the sym5-CWT amplitudes at 287.75 nm for LAM and 299.15 nm for ZID. The regression equations and related statistical results were indicated in Table 1. The concentrations of LAM and ZID in their samples were calculated using the obtained regression equations.

As described in the mexh-CWT and sym5-CWT applications, the db10-CWT method with the scaling factor a=450 was applied to the UV spectra data vectors of pure LAM and ZID, and their samples in the wavelet domain. The sym10-CWT spectra of LAM and ZID were illustrated in Fig. 1D. The calibration graphs for the analyzed drugs were obtained by measuring the sym10-CWT amplitudes at 287.60 nm for LAM and 275.75 nm for ZID in the sym10-CWT spectra. The quantity of LAM and ZID in samples was analyzed using the calculated calibration equations.

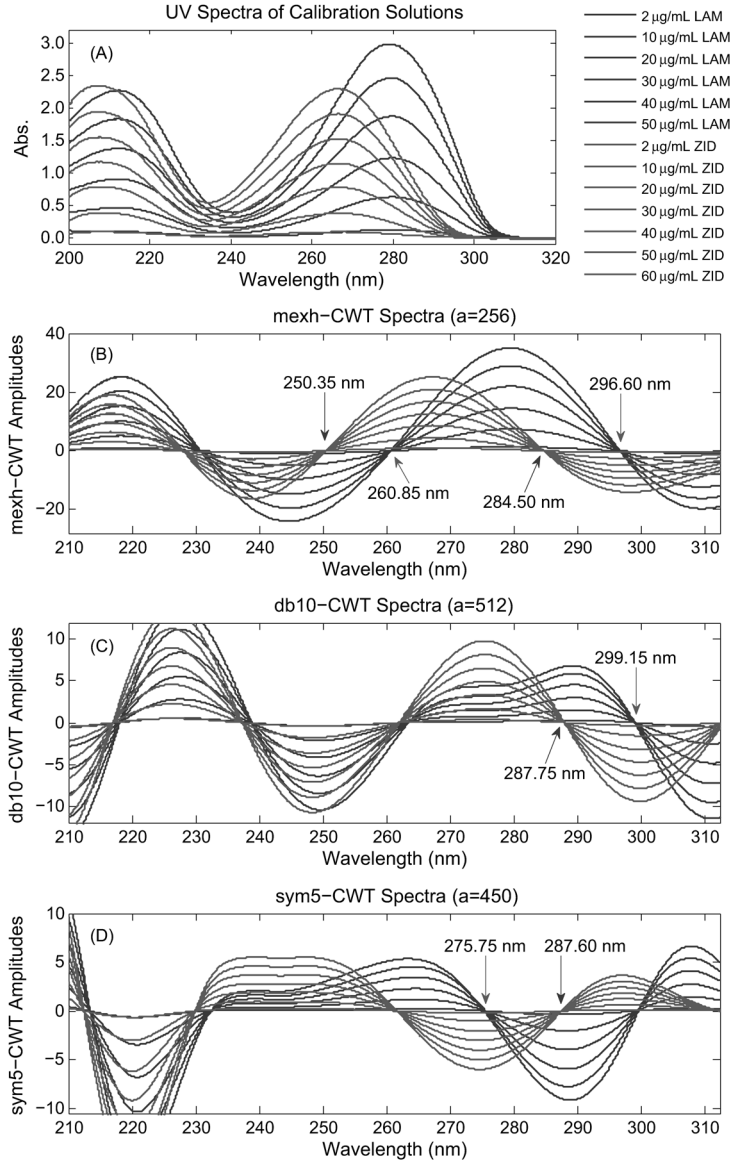


Fig. 1. The UV Spectra of the Calibration Samples (A), mexh-CWT Spectra (B), db10-CWT Spectra (C) and sym5-CWT Spectra (D) Obtained from the UV Spectra of the Calibration Solutions

Table 1. Least Squares Regression Analysis and Statistical Results Obtained by the Improved mexh-CWT, db10-CWT and sym5-CWT Methods

	λ (nm)	Range (μg/mL)	Regression function	r	S.E. (m)	S.E. (n)	S.E. (r)	LOD	LOQ
mexh	250.35	2.0–50.0	$S=-0.4094C_{LAM}-0.1376$	0.9997	4.94×10^{-3}	2.96×10^{-2}	2.01×10^{-1}	0.53	1.77
	284.50	2.0–50.0	$S=0.6279C_{LAM}+0.2832$	0.9994	1.11×10^{-2}	3.37×10^{-2}	4.53×10^{-1}	0.39	1.32
	260.85	2.0–60.0	$S=0.3353C_{ZID}+0.1285$	1.0000	1.27×10^{-3}	2.56×10^{-2}	6.59×10^{-2}	0.61	2.02
	296.60	2.0–60.0	$S=-0.2272C_{ZID}-0.2044$	0.9999	1.57×10^{-3}	1.67×10^{-2}	8.14×10^{-2}	0.58	1.94
db10	287.75	2.0–50.0	$S=0.1360C_{LAM}+0.1471$	0.9975	4.85×10^{-3}	1.19×10^{-2}	1.97×10^{-1}	0.65	2.15
	299.15	2.0–60.0	$S=-0.1562C_{ZID}-0.0275$	1.0000	4.94×10^{-4}	1.08×10^{-2}	2.56×10^{-2}	0.55	1.83
sym5	287.60	2.0–50.0	$S=-0.1824C_{LAM}-0.2078$	0.9976	6.27×10^{-3}	1.70×10^{-2}	2.55×10^{-1}	0.68	2.28
	275.75	2.0–60.0	$S=-0.0984C_{ZID}-0.0515$	0.9999	4.55×10^{-4}	5.40×10^{-3}	2.36×10^{-2}	0.44	1.45

S is the CWT amplitudes of the UV absorption spectra. C_{LAM} C_{ZID} are the concentration of analyzed drugs (μg/mL). S.E. (m) is the standard error of straight-line slope. S.E. (n) is the standard error of straight-line intercept. S.E. (r) is the standard error of the regression equation coefficient. LOD is the limit of detection (μg/mL). LOQ is the limit of quantitation (μg/mL).

Table 2. Recovery Results Obtained by Applying the Improved mexh-CWT, db10-CWT and sym5-CWT Methods to the LAM–ZID Mixture Samples

Predicted conc. (μg/mL)									
Binary mixture (μg/mL)		mexh-CWT				db10-CWT		sym5-CWT	
		LAM		ZID		LAM	ZID	LAM	ZID
LAM	ZID	250.35 nm	284.5 nm	260.85 nm	296.6 nm	287.75 nm	299.15 nm	287.6 nm	275.75 nm
2	48	2.06	2.02	48.91	49.03	2.05	48.84	2.01	48.91
10	48	10.58	10.5	47.57	47.58	10.22	47.54	9.76	47.66
20	48	20.57	20.65	47.95	47.51	20.88	48.21	20.45	46.82
30	48	29.45	29.95	46.9	47.25	29.74	45.86	30.62	47.07
40	48	39	38.48	47.44	47.27	38.92	47.02	39.06	49.78
50	48	48.68	49.6	50.28	47.24	49	48.14	48.9	48.06
24	2	23.54	23.64	2.07	1.98	23.86	1.98	23.3	1.97
24	10	24.16	24.29	9.57	10.14	24.95	10.08	24.8	10.24
24	20	24.59	24.71	19.87	20.42	24.34	19.57	24.99	20.84
24	30	24.49	24.51	29.37	29.67	25	30.1	24.57	30.06
24	40	24.52	24.62	40.07	39.93	24.75	40.35	24.66	39.55
24	50	24.19	24.53	49.61	49.58	23.95	49.33	24.79	47.94
24	60	23.53	24.36	58.71	58.52	23.28	58.86	25.59	60.39
24	48	24.01	24.25	47.19	47.41	23.89	47.34	24.36	45.95
Recovery (%)									
		mexh-CWT				db10-CWT		sym5-CWT	
		LAM		ZID		LAM	ZID	LAM	ZID
		250.35 nm	284.5 nm	260.85 nm	296.6 nm	287.75 nm	299.15 nm	287.6 nm	275.75 nm
		103.0	101.0	101.9	102.1	102.5	101.8	100.5	101.9
		105.8	105.0	99.1	99.1	102.2	99.0	97.6	99.3
		102.9	103.3	99.9	99.0	104.4	100.4	102.3	97.5
		98.2	99.8	97.7	98.4	99.1	95.5	102.1	98.1
		97.5	96.2	98.8	98.5	97.3	98.0	97.7	103.7
		97.4	99.2	104.8	98.4	98.0	100.3	97.8	100.1
		98.1	98.5	103.5	99.0	99.4	99.0	97.1	98.5
		100.7	101.2	95.7	101.4	104.0	100.8	103.3	102.4
		102.5	103.0	99.4	102.1	101.4	97.9	104.1	104.2
		102.0	102.1	97.9	98.9	104.2	100.3	102.4	100.2
		102.2	102.6	100.2	99.8	103.1	100.9	102.8	98.9
		100.8	102.2	99.2	99.2	99.8	98.7	103.3	95.9
		98.0	101.5	97.9	97.5	97.0	98.1	106.6	100.7
		100.0	101.0	98.3	98.8	99.5	98.6	101.5	95.7
	Mean	100.6	101.2	99.6	99.4	100.9	99.2	101.4	99.8
	S.D.	2.56	2.22	2.40	1.42	2.58	1.63	2.87	2.63
	R.S.D.	2.54	2.19	2.41	1.43	2.56	1.64	2.83	2.63

S.D., standard deviation; R.S.D., relative standard deviation.

Validity of CWT Signal Processing Methods In the application of three different CWT signal processing methods, a good linearity for LAM and ZID in the working range of 2.0–50.0 μg/mL and 2.0–60.0 μg/mL, respectively, was observed from the regression coefficients indicated in Table 1. The limit of quantitation (LOD, signal/noise=3) and limit of detection (LOQ, signal/noise=10) were calculated using standard deviation of the linear regression curves of the related drugs.

The validity and ability of the improved mexh-CWT, sym5-CWT and db10-CWT were tested by analyzing the validation samples containing binary mixtures of LAM and ZID in the different concentration levels. Percent recovery, standard deviation and relative standard deviation for both drugs were depicted in Table 2. These values demonstrate satisfactory accuracy and precision of the proposed CWT signal processing methods.

Plot of the Dissolution Profiles In the dissolution testing procedure, USP Apparatus II-Paddle method was used to collect the dissolution samples during 60 min. The pH 1.2 buffer solution was used as dissolution medium. As explained above, the UV absorption spectra of the collected dissolution samples were recorded and processed by the mexh-CWT, sym5-CWT and db10-CWT approaches. The calculated dissolution data were used to obtain the dissolution profiles of LAM and ZID in tablets.

The dissolution profiles obtained by applying the mexh-CWT approach to the commercial LAM–ZID tablets were presented in Figs. 2A and B. In these dissolution profiles, it was concluded that the mean percentage and its correspond-

Table 3. Dissolution Data Obtained by Applying the Improved mexh-CWT Method to the Dissolution Samples

mexh-CWT method (a=256)								
Time (min)	LAM (λ=250.35 nm)		ZID (λ=296.60 nm)		LAM (λ=284.50 nm)		ZID (λ=260.85 nm)	
	Dissolved (%)	CL	Dissolved (%)	CL	Dissolved (%)	CL	Dissolved (%)	CL
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	44.07	9.54	43.68	16.17	43.43	9.58	42.49	16.71
5	85.63	5.75	88.92	10.35	85.20	5.8	85.47	10.43
10	93.02	3.65	93.62	7.08	92.23	3.73	93.81	6.90
15	94.56	2.72	97.73	3.77	94.00	2.82	96.75	4.29
20	95.14	2.36	97.82	3.44	94.66	2.35	97.31	3.44
30	96.36	0.92	98.39	1.99	95.91	0.87	98.14	2.07
45	98.19	0.8	99.16	1.14	97.94	0.71	99.41	1.17
60	98.45	0.74	98.85	1.08	98.22	0.77	99.04	0.71

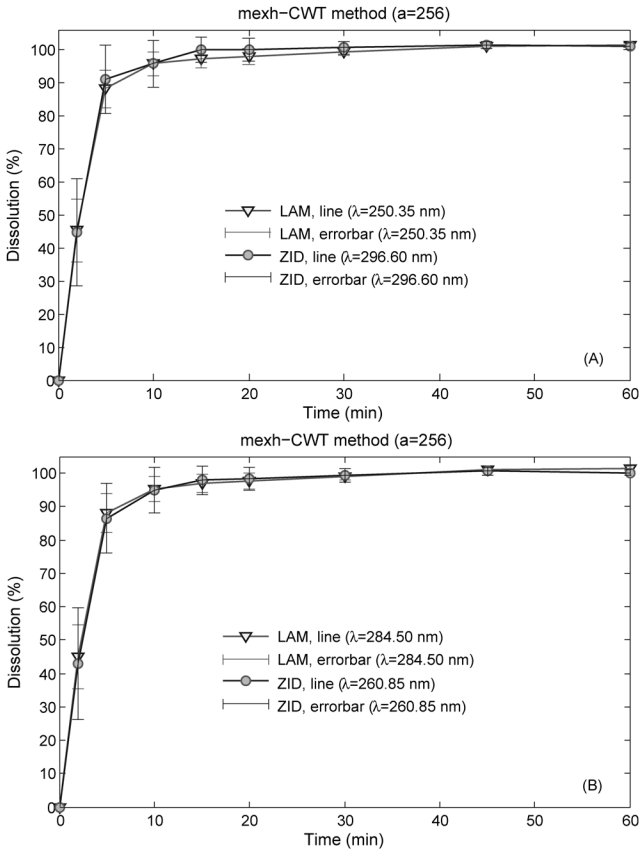


Fig. 2. Dissolution Profiles of LAM and ZID in Tablets by Applying the mexh-CWT Method at 250.35 nm and 296.60 nm (A) and 284.50 nm and 260.85 nm (B), Respectively

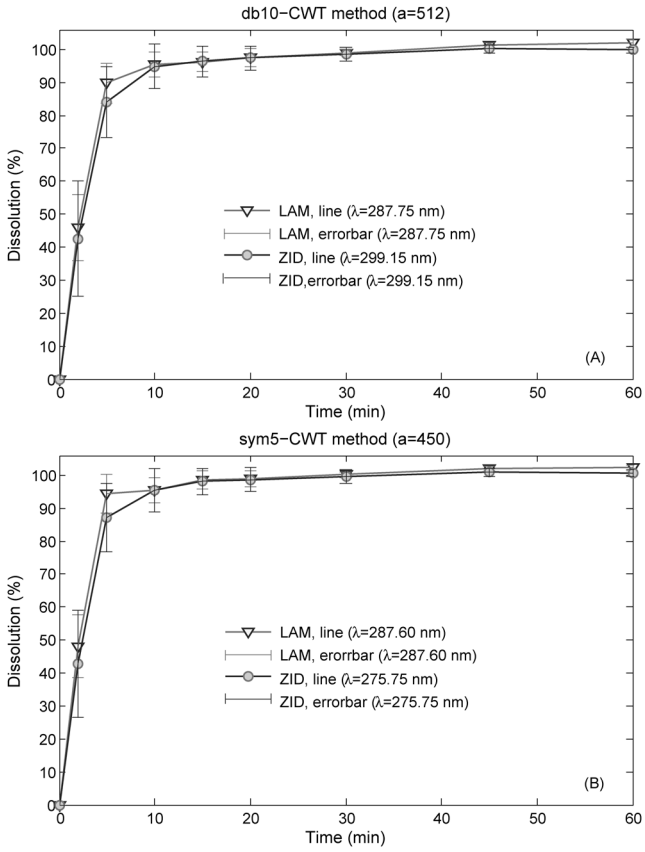


Fig. 3. Dissolution Profiles of LAM and ZID in Tablets by Applying the db10-CWT (A) and sym5-CWT (B) Methods

ing confidence limit of the dissolved drug within 10 min were found to be 93.02% and 3.65% for LAM and 93.62% and 7.08% for ZID (see Table 3) and 92.23% and 3.73% for LAM and 93.81% and 6.90% for ZID (see Table 3), respectively.

In the application of db10-CWT approach to LAM–ZID tablets, the dissolution profiles for both LAM and ZID were presented in Fig. 3A. As it can be seen from Table 4, 90.31% with confidence limit 3.85% of LAM and 93.98% with confidence limit 6.75% of ZID from tablets were dissolved within 10 min.

In the case of the sym5-CWT method, the dissolution profiles of the related drugs were displayed in Fig. 3B. The sym5-CWT application reveals that the percent dissolution and its confidence level of the dissolved drug in the first 10 min of the test were 91.96% and 3.95% for LAM and 93.87% and 6.50% for ZID, respectively, as presented in Table 4. All the dissolution results indicated that approximately 90% of both drugs were dissolved within the first 10 min.

Analysis of Commercial Tablets Analysis of the commercial tablets containing LAM and ZID was performed by using the improved mexh-CWT, sym5-CWT and db10-CWT methods. The determination results were presented in Table 5. A good coincidence between these results was observed for the ability and applicability of the proposed methods for the simultaneous determination of LAM and ZID in pharmaceutical tablet samples. Analysis results showed that the improved

Table 4. Dissolution Data Obtained by Applying the Improved db10-CWT and sym5-CWT Methods to the Dissolution Samples

Time (min)	db10-CWT method (a=512)				sym5-CWT method (a=450)			
	LAM (λ=287.75 nm)		ZID (λ=299.15 nm)		LAM (λ=287.60 nm)		ZID (λ=275.75 nm)	
	Dissolved (%)	CL	Dissolved (%)	CL	Dissolved (%)	CL	Dissolved (%)	CL
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	43.48	9.97	42.15	17.44	46.28	9.54	42.06	16.26
5	85.03	6.04	83.29	10.61	90.94	5.81	85.58	10.40
10	90.31	3.85	93.98	6.74	91.96	3.95	93.87	6.50
15	91.19	2.94	95.54	4.72	94.85	2.72	96.39	4.10
20	92.35	2.70	96.59	3.75	95.31	2.33	96.91	3.63
30	93.82	0.85	97.72	2.18	96.47	0.73	97.74	2.08
45	96.00	0.67	99.34	1.26	98.19	0.74	99.08	1.13
60	96.49	1.30	98.97	0.77	98.47	0.73	98.92	1.04

Table 5. Determination Results Obtained by Applying the Improved mexh-CWT, db10-CWT and sym5-CWT Methods to the Commercial Tablet Samples

No.	Mexh-CWT				db10-CWT		sym5-CWT	
	LAM		ZID		LAM	ZID	LAM	ZID
	250.35 nm	284.50 nm	260.85 nm	296.60 nm	287.75 nm	299.15 nm	287.60 nm	275.75 nm
1	151.2	152.0	302.3	301.0	152.0	302.1	152.7	298.5
2	150.7	151.4	300.7	300.0	151.3	300.2	152.3	301.5
3	149.2	150.0	298.5	297.9	150.5	298.2	150.5	296.4
4	149.4	153.8	299.2	297.3	150.2	298.8	150.7	295.7
5	152.8	153.8	306.1	304.9	152.9	304.4	154.9	301.1
6	150.5	151.4	300.7	299.3	150.5	299.8	152.0	296.8
7	149.9	152.8	299.1	296.0	150.9	300.0	150.5	296.0
8	149.1	150.1	298.2	296.1	149.3	297.5	150.2	294.7
9	151.1	152.2	302.3	302.2	151.7	300.2	153.4	298.4
10	152.8	153.8	305.4	304.0	152.7	304.0	154.2	301.5
Mean	150.7	152.1	301.2	299.9	151.2	300.5	152.1	298.0
S.D.	1.35	1.43	2.76	3.13	1.14	2.31	1.66	2.56
R.S.D.	0.9	0.94	0.92	1.04	0.76	0.77	1.09	0.86
S.E.	0.43	0.45	0.87	0.99	0.36	0.73	0.52	0.81
CL	0.84	0.89	1.71	1.94	0.71	1.43	1.03	1.59

S.D., standard deviation; R.S.D., relative standard deviation; S.E., standard error; CL: confidential limit (p=0.05).

CWT signal processing methods were found to be specific for the simultaneous analysis of LAM and ZID drugs in their combined tablets without interference from the above mentioned inactive ingredients. The proposed mexh-CWT, sym5-CWT and db10-CWT methods allowed the selective quantitative analysis of each drug in presence of the other without any interference proving their selectivity and ability to resolve a combined tablet dosage form of the analyzed drugs.

The tablet assay results obtained by the applying three methods to the commercial preparations were statistically compared with each other by using one way ANOVA test. From statistical tables, the theoretical F-value at a 5% level of significance for n_1=3 and n_2=32 is given as 2.90, which corresponds to F-critical value in Table 6. In practice, the calculated F-values were found to be 2.36 for LAM and 1.99 for ZID. As it can be seen from Table 6, the one-way ANOVA test shows that there are no significant differences between the results obtained by mexh-CWT, sym5-CWT and db10-CWT approaches.

Conclusion

In this study, we have introduced new signal processing methods, mexh-CWT, sym5-CWT and db10-CWT approaches, for the simultaneous quantitative analysis and dissolution testing of LAM and ZID in tablets. The experiments were carried out without any chemical pre-treatment or any preliminary separation step. The assay results obtained in this study strongly encourage us to apply these CWT approaches for a routine analysis and quality control, and dissolution testing of the pharmaceutical tablet formulation containing LAM and ZID drugs.

New hybrid approaches based on the wavelets with zero crossing technique offers new possibilities and alternative ways for the simultaneous quantitative resolution and dissolution testing of combined pharmaceutical preparations in the presence overlapping spectra of analyzed drugs without using prior separation step, instead of the conventional analysis methods mentioned in the introduction section.

As a consequence, the improved mexh-CWT, sym5-CWT and db10-CWT methods can be applied to the routine analysis and dissolution testing of LAM and ZID in tablets.

Table 6. Statistical Test Results Obtained by Applying One-Way ANOVA Test to the Tablet Analysis Results Provided by the Improved mexh-CWT, db10-CWT and sym5-CWT Methods

Source of variation	Drug	Between groups	Within groups	Total
SS	LAM	15.56	70.35	85.91
	ZID	48.14	258.08	306.21
df	LAM	3.00	32.00	35.00
	ZID	3.00	32.00	35.00
MS	LAM	5.19	2.20	
	ZID	16.05	8.06	
F-Calculated	LAM	2.36		
	ZID	1.99		
p-Value	LAM	0.09		
	ZID	0.14		
F-Critical	LAM	2.90		
	ZID	2.90		

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

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