Application of the Wavelet Method for the Simultaneous Quantitative Determination of Benazepril and Hydrochlorothiazide in Their Mixtures

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The discrete and continuous wavelet transforms were applied to the overlapping signal analysis of the ratio data signal for simultaneous quantitative determination of the title subject compounds in samples. The ratio spectra data of the binary mixtures containing benazepril (BE) and hydrochlorothiazide (HCT) were transferred as data vectors into the wavelet domain. Signal compression, followed by a 1-dimension continuous wavelet transform (CWT), was used to obtain coincident transformed signals for pure BE and HCT and their mixtures. The coincident transformed amplitudes corresponding to both maximum and minimum points allowed construction of calibration graphs for each compound in the binary mixture. The validity of CWT calibrations was tested by analyzing synthetic mixtures of the investigated compounds, and successful results were obtained. All calculations were performed within EXCEL, C++, and MATLAB6.5 softwares. The obtained results indicated that our approach was flexible and applicable for the binary mixture analysis.

Recently, many analytical methods, namely numerical and graphical methods, have been developed for the simultaneous determination of 2 or more compounds in complex mixtures. These methods contain various disadvantages, e.g., diminished peak amplitude in higher-order derivative spectrophotometry or complex calculations in chemometrics methods. For these reasons, analytical chemistry needs new approaches for mixture analysis without using a separation step.

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One of those new and promising methods is the wavelet transform (WT) method (1–3). The WT method can be classified into 2 categories, continuous wavelet transform (CWT) and discrete wavelet transform (DWT). Very recently, WT was subjected to an intense debate and some interesting applications were found. WT was applied to spectroscopic analysis (4); removing nonconstant, varying spectroscopic background in multivariate calibration (5); analyzing and an oscillating chemical system (6); background correction in inductively coupled plasma atomic emission spectrometry (7); and multicomponent analysis of electrochemical signals (8).

WT overcomes the limitations of Fourier series, and it becomes very useful in signal analysis especially for denoising and compressing data (9). In analytical spectroscopy, WT is applicable because we are dealing with absorbance spectra. These spectra can be considered as a 1-dimensional signal, and they can be analyzed within 1-dimensional WT. WT becomes a new and powerful mathematical tool for solving the analytical problems concerning the overlapping signals in the complex mixture analyzed (10).

In this study, a new application of WT to ratio spectra data (11–14) for the quantitative analysis of binary mixtures is proposed. Firstly, DWT is used for compression of the ratio absorbance data in the wavelet domain. Secondly, CWT was applied to the analyzed–compressed signals of the ratio absorbance data in the above-mentioned wavelet domain. As a result of this application, the coincident continuous wavelet spectra were obtained by giving a maximum and minimum for pure compound and its mixture with other compounds.

The calibration graphs were obtained by measuring the continuous wavelet amplitude at a maximum and a minimum in the working wavelet domain. Calibration lines for pure analytes were tested by using a synthetic mixture of analyzed compounds. Afterwards, this method was applied to real samples comprising 2 pharmaceutical formulations.

Theoretical Aspects

The Wavelet Method

The fundamental idea behind wavelets is to analyze according to scale. Indeed, some researchers in the wavelet

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field believe that by using wavelets, one is adopting a whole new mindset or perspective in processing data. Wavelets are functions that satisfy certain mathematical requirements and are used in representing data or other functions. This idea is not new. J. Fourier discovered in the early 1800s that he could superpose sines and cosines to represent other functions. However, in wavelet analysis, the scale that one uses in looking at data plays a special role. Wavelet algorithms process data at different scales or resolutions. If we look at a signal with a large window, we would notice gross features. Similarly, if we look at a signal with a small window, we would notice small discontinuities.

Compression Processes Using Discrete Wavelet Transform

A wavelet compression consists of the following steps: (*1*) Perform a wavelet transform of the signal. (*2*) Set all wavelet coefficients, which are insignificant, to 0, i.e., which lie below some threshold value. (*3*) Transmit only the significant, non-0 values of the transform obtained from Step 2. This should be a much smaller data set than the original signal.

Continuous Wavelet Transform

A mixture of frequencies, shown by its own Fourier transform, composes a wavelet. Wavelet coefficients necessarily refer to this mixture of frequencies. By definition, a wavelet has an integral of 0, half the area it surrounds is positive, the other is negative. If we multiply the wavelet by a constant, it changes the positive and the negative parts equally and the integral remains 0.

Wavelet is expressed as a series of functions $\Psi_{a,b}(\lambda)$ having the following forms:

$$\Psi_{a,b}(\lambda) = \frac{1}{\sqrt{|a|}}\Psi\left(\frac{\lambda - b}{a}\right) \quad a \neq 0, \quad a, b \in R \qquad (1)$$

Here, a represents the scale parameter, which is a variable used to control the scaling; b represents the translation parameter controlling the translation; and R is the domain of real numbers. In other words, we have a set of functions $\Psi_{a,b}(\lambda)$ obtained from a mother wavelet $\Psi(\lambda)$ by scaling (or dilatation) and shifting (or translation).

For a wavelength number-varying signal (λ), WTs contain the computation of coefficients obtained from the inner products of the signal and a family of wavelets. CWT of f (λ) is defined as:

$$\mathrm{CWT}\{f(\lambda); a, b\} = \int_{\infty}^{\infty} f(\lambda)\,\psi^{*}_{a,b}(\lambda)\,d\lambda = \langle f(\lambda), \psi_{a,b}\rangle \qquad (2)$$

Here the superscript * represents the complex conjugate, and $\langle f(\lambda), \psi_{a,b}\rangle$ denotes the inner product of function f (λ) onto the wavelet function $\Psi_{a,b}(\lambda)$.

In this paper, we will focus on the Gaussian wavelets $f_a(\lambda) = \frac{1}{2}(\pi a) - \frac{1}{2}\, e^{-\frac{\lambda^2}{4\alpha}}$ and its derivatives of order 4 (gaus4), as well as Coiflets wavelets. Coiflets are another variation on Daubechies wavelets. They are so named by Daubechies because Coifman suggested the construction of orthonormal wavelet basis with vanishing moment conditions both for φ as well as for ψ (for more details *see* ref. 2). The idea is to find the form of $m_o(\omega)$ that satisfies the orthogonality condition and both moment conditions for φ and ψ. These moment conditions are

$$\int_{\infty}^{\infty}\varphi(f)dt = 1, \ \int_{\infty}^{\infty}\gamma\varphi(f)dt = 0, \quad \text{for } l = 1,\ldots\ldots,N-1 \qquad (3)$$

These 2 moment conditions translate into the following conditions on $m_0(\omega)$:

$$m_0^{(1)}(\pi) = 0, \ m_0(0) = 1, \ m_0^{(1)}(0) = 0, \ \text{for } l = 1,2,\ldots\ldots,N-1 \qquad (4)$$

It can be shown that the following form of $m_0(\omega)$ fulfils both the moment conditions given above

$$m_0(\omega) = \left(\cos^2\frac{\omega}{2}\right)^K\left[\sum_{k=0}^{K-1}\binom{K-1+k}{k}\left(\sin^2\frac{\omega}{2}\right)_k + \left(\sin^2\frac{\omega}{2}\right)^K f(\omega)\right] \qquad (5)$$

where $2K = N$ and $f(\omega)$ is chosen to be

$$f(\omega) = \sum_{n=0}^{2K-1} f_n e^{in\omega} \qquad (6)$$

The coefficients $\{f_\omega\}$ are determined by requiring $m_0(\omega)$ given in Equation 6 to satisfy the orthogonality condition. Among 5 coiflets family we retained only coif2 because it corresponds to our requirements mentioned above.

Application of CWT to the Ratio Data Signal

Let us assume that a mixture contains 2 compounds, X and Y. If Beer's law is valid for these 2 compounds over the used wavelength range and if the path length is 1 cm, then the following equation can be written for the absorbance of a mixture of X and Y:

$$A_{m,\lambda i} = \alpha_{X,\lambda i} C_X + \beta_{Y,\lambda i} C_Y \qquad (7)$$

Here $A_{m,\lambda i}$ denotes the absorbance of the mixture at wavelength λi, $\alpha_{X,\lambda i}$ and $\beta_{X,\lambda i}$ are the absorptivities of X and Y compounds, respectively, and C_X and C_Y are the concentrations of compounds X and Y, respectively.

The spectrum of the standard solution of one of the compounds in the same binary mixture is described by the following equation:

$$A_{m,\lambda i} = \beta_{Y,\lambda i} C_Y^0 \qquad (8)$$

If we divide Equation 7 by Equation 8 , the ratio spectrum is obtained as

$$\frac{A_{m,\lambda i}}{\beta_{Y,\lambda i} C_Y^0} = \frac{\alpha_{X,\lambda i} C_X}{\beta_{Y,\lambda i} C_Y^0} + \frac{\beta_{Y,\lambda i} C_Y}{\beta_{Y,\lambda i} C_Y^0} \qquad (9)$$

or, more simply in the following form

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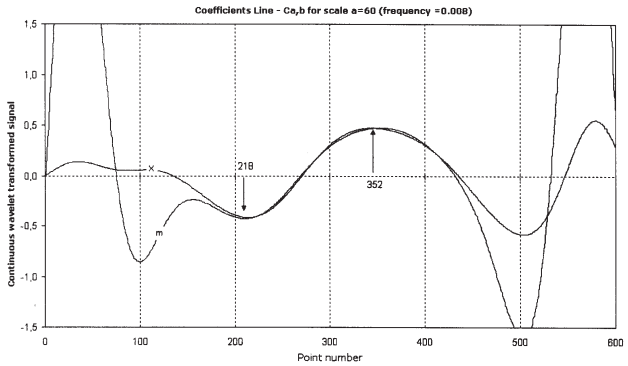


Figure 1. Continuous wavelet transformed coincident spectrum of 16 μg/mL BE (x) and its binary mixture (m) with 20 μg/mL HCT (the transformed coincident spectrum of data signals obtained by applying a 6-level 1-dimensional db10 wavelet compression and gaus4 CWT to the ratio spectra data of $\frac{16\ \mu g/mL\ BE + 20\ \mu g/mL\ HCT}{16\ \mu g/mL\ HCT}$ (m) and $\frac{16\ \mu g/mL\ BE}{16\ \mu g/mL\ HCT}$ (x) in the wavelet domain).

$$\frac{A_{m,\lambda i}}{\beta_{Y,\lambda i}\, C_Y^0} = \frac{\alpha_{X,\lambda i}\, C_X}{\beta_{Y,\lambda i}\, C_Y^0} + \frac{C_Y}{C_Y^0} \qquad (10)$$

The data vector of the ratio signal $\frac{A_{m,\lambda i}}{\beta_{Y,\lambda i}\, C_Y^0}$ corresponding to Equation 10 is transferred into wavelet domain. Let us denote the above signal as $\Psi_{m,Pi}\frac{1}{C_Y^0}$ and $\frac{\alpha_{X,\lambda i}}{\beta_{Y,\lambda i}\, C_Y^0}$ with $\Psi_{X,Pi}\frac{1}{C_Y^0}$.

If the CWT is applied to Equation 10, and taking into account that CWT of the constant $\frac{C_Y}{C_Y^0}$ is 0, we obtain

$$CWT\left[\Psi_{m,Pi}\right]\frac{1}{C_Y^0} = CWT\left[\Psi_{X,Pi}\right]\frac{C_X}{C_Y^0} \qquad (11)$$

Equation 11 represents the continuous wavelet spectrum of the mixture containing compounds X and Y. The continuous wavelet amplitudes corresponding to minimum and maximum depend only to the values of C_X and C_Y^0, and they are independent of the C_Y^0 values in the binary mixture. Equation 11 is illustrated in Figures 1 and 2 in the case of a binary mixture (m) of benazepril (BE; X) and hydrochlorothiazide (HTC; Y). The continuous wavelet-transformed coincident amplitudes (CWTCA) of pure BE and its binary mixture with HCT were corresponded to a minimum at 218 point number and a maximum at 352 point number by using gaus4 CWT (Figure 1). By using the same CWT, the transformed coincident amplitudes of HCT and its mixture with BE were corresponded to a maximum at 557 point number and a minimum at 620 point number (Figure 2).

In this method, the concentration C_X in the binary mixture is proportional to CWT amplitudes corresponding to minimum and maximum points in the wavelet domain. The calibration graphs for pure compound X at increasing concentration are obtained by measuring the continuous wavelet amplitudes at the minimum and maximum points in the wavelet domain. Making use of the calibration graphs, we can determine C_X concentration corresponding to compound X in binary mixture with Y. The concentration of compound Y is determined by an analogous procedure.

Experimental

Instruments

A Shimadzu (Kyoto, Japan) UV-160 double-beam UV-Vis spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and an HP DeskJet 600 printer were used to record the absorption spectra. The application of WT to regression and statistical analyses was achieved by using C++, MATLAB 6.5, and EXCEL softwares.

To make the wavelet compression, a program in C++ was prepared. The signals obtained were transferred into wavelet domain and subjected to CWT.

Commercial Tablet Formulation

Two commercial pharmaceutical formulations, Cibadrex® Divitab 5/6.25 (I) and 10/12.5 (II) tablets (produced by Novartis Pharmaceuticals Turkey, Batch Nos. 13 and 18, respectively) containing 5 and 10 mg BE, and 6.25 and 12.50 mg HCT were analyzed by using the proposed CWT approaches.

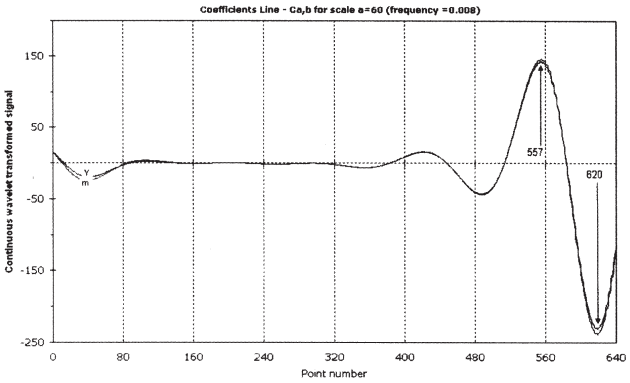


Figure 2. Continuous wavelet transformed coincident spectrum of 20 μg/mL HCT (y) and its binary mixture (m) with 16 μg/mL BE (the transformed coincident spectrum of data signals obtained by applying a 6-level 1-dimensional db10 wavelet compression and gaus4 CWT to the ratio spectra data of $\frac{16\ \mu g/mL\ BE + 20\ \mu g/mL\ HCT}{24\ \mu g/mL\ BE}$ (m) and $\frac{16\ \mu g/mL\ HCT}{24\ \mu g/mL\ VE}$ (x) in the wavelet domain).

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Standard Solutions

Stock solution of 100 mg/100 mL BE and HCT was prepared in 0.1M NaOH. A standard series of the solutions containing 12–36 μg/mL BE and 10–22 μg/mL HCT was prepared from the stock solutions. A validation set consisting of 10 synthetic mixture solutions in the working range of 12–36 μg/mL BE and 10–22 μg/mL HCT was prepared by using the same stock solutions. All solutions were prepared freshly and protected from light.

Tablet Analysis

Twenty tablets were accurately weighed and powdered in a mortar and pestle. An amount equivalent to 1 tablet was dissolved in 0.1M NaOH in a 100 mL volumetric flask by sonication. The solution was filtered into a 100 mL volumetric flask through Whatman No. 42 filter paper. This procedure was utilized for Cibadrex Divitab 5/6.25 (I) and 10/12.5 (II) tablets. Tablet solutions (I) and (II) were diluted to the working concentration range 1/3.125 and 1/6.25 in a 25 mL volumetric flask with 0.1M NaOH, respectively. The optimized 2 wavelet families were successfully applied to the analysis of 2 pharmaceutical tablet formulations.

Results and Discussion

The essential aim of this study was to apply CWT to the simultaneous quantitative analysis of mixtures containing 2 target compounds. The CWT application was based on the use of the coincident continuous wavelet amplitudes of a pure active compound and its binary mixture in the wavelet domain. This procedure contains 3 main steps as indicated in the following sequence: *Step 1 treatment*.—(*a*) Record absorption spectra; (*b*) ratio procedure of the absorption spectra. *Step 2 treatment*.—(*a*) Compression wavelet analysis; (*b*) CWT. *Step 3 treatment*.—Calibration graph.

Recorded Absorption Spectra

The absorption spectra of HCT and BE in the concentration range of 10–22 and 12–36 μg/mL, respectively, were recorded and stored in the 215–355 nm wavelength range as indicated in Figure 3. The absorption spectra of the synthetic binary mixture and 2 pharmaceutical tablet samples containing the 2 active compounds were also plotted in the same wavelength range. These recorded spectra were used for the following sub-procedures.

Ratio Procedure of the Absorption Spectra

The main problem was to find the optimum divisor concentration. Generally, a good linearity between concentration and signal is provided by the divisor spectrum corresponding to the average amount in the working concentration range. In application of the CWT to the transferred (analyzed) ratio spectra data vector, in order to select a divisor for the given concentration in the working concentration range some divisor concentrations were tested. The standard spectrum of 16 μg/mL HCT solution for determining BE and of 24 μg/mL BE solution for the determination of HCT in their binary mixture was found suitable for the ratio procedure and the application of DWT and CWT.

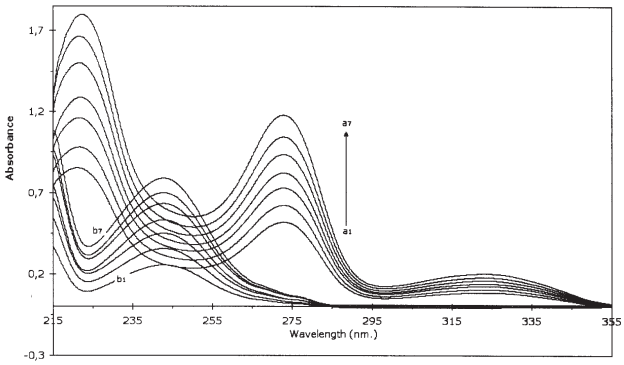


Figure 3. Absorption spectra of (a1) 10 μg/mL, (a2) 12 μg/mL, (a3) 14 μg/mL, (a4) 16 μg/mL, (a5) 18 μg/mL, (a6) 20 μg/mL, (a7) 22 μg/mL HTC and (b1) 12 μg/mL, (b2) 16 μg/mL, (b3) 20 μg/mL, (b4) 24 μg/mL, (b5) 28 μg/mL, (b6) 32 μg/mL, (b7) 36 μg/mL BE in 0.1M NaOH.

The absorption spectra of 10–22 μg/mL HCT were divided by the standard spectrum of 24 μg/mL BE (Figure 4A), and the obtained ratio spectra data consisting of 640 point values were transferred into the wavelet domain of 0–640 (Figure 4B). In a similar way, the absorption spectra of 12–36 μg/mL BE were divided by the standard spectrum of 16 μg/mL HCT (Figure 5A) and their ratio spectra data were transferred in the wavelet domain of 0–640 point number (Figure 5B).

Compression Wavelet Analysis

As we could see, our transferred signal (Figures 4B, 5B) was not smooth, so we decided to make it so by using the compression procedure. This aspect is important because a smooth signal is involved in obtaining the coincident continuous wavelet signals. In our study, all transferred ratio data signals of HCT and BE were compressed by using a 6-level of daub10 discrete wavelet (Figures 4C and 5C). The retained energy was found to be 98.88, 98.68, and 98.66% for tablet, mixture, and pure value of HCT and 99.99, 99.98, and 99.98% for tablet, mixture, and pure value of BE, respectively.

Continuous Wavelet Transform

The optimal families of CWT were identified in our study. To reach this objective, various CWT were tested for obtaining coincident continuous wavelet signals. We observed that the scale parameter plays an important role in this investigation. CWT methods, namely gaus4 corresponding to the scale parameter a = 60 (frequency = 0.008) and coif2 corresponding to a = 75 (frequency = 0.010),

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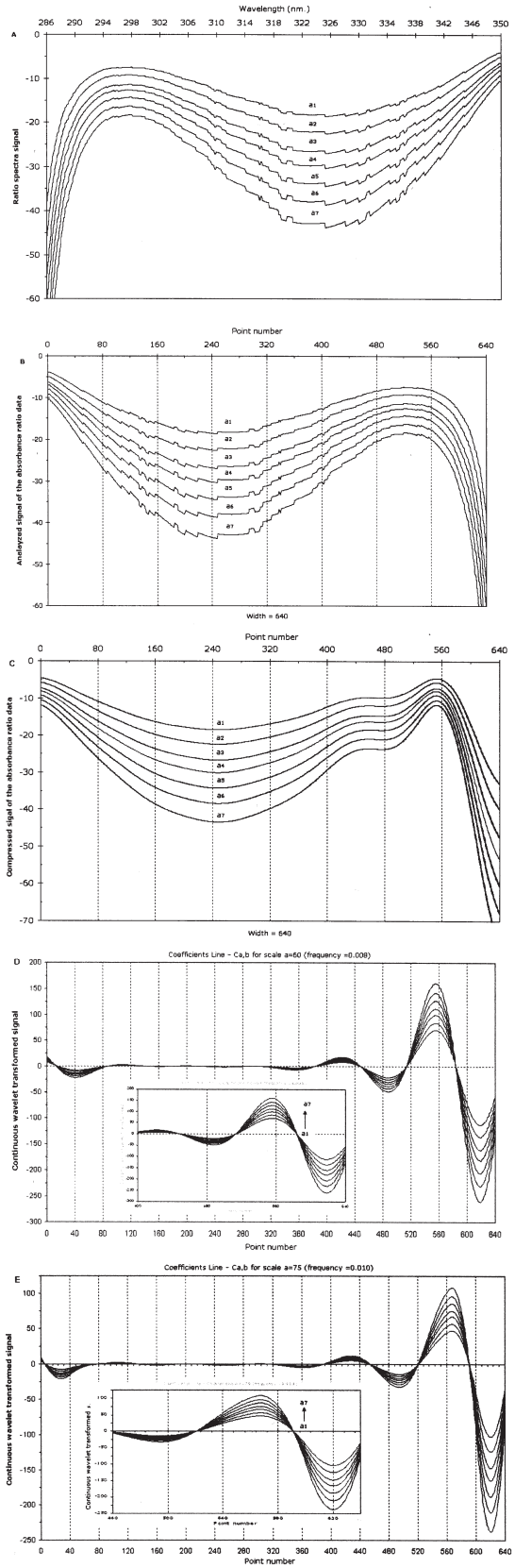


Figure 4. Ratio spectrum (A), analyzed spectrum (B), compressed spectrum (C), gaus4 CWT spectrum (D), and coif2 CWT spectrum (E) of: (a1) 10 μg/mL, (a2) 12 μg/mL, (a3) 14 μg/mL, (a4) 16 μg/mL, (a5) 18 μg/mL, (a6) 20 μg/mL, (a7) 22 μg/mL HTC using 16 μg/mL BE as a divisor.

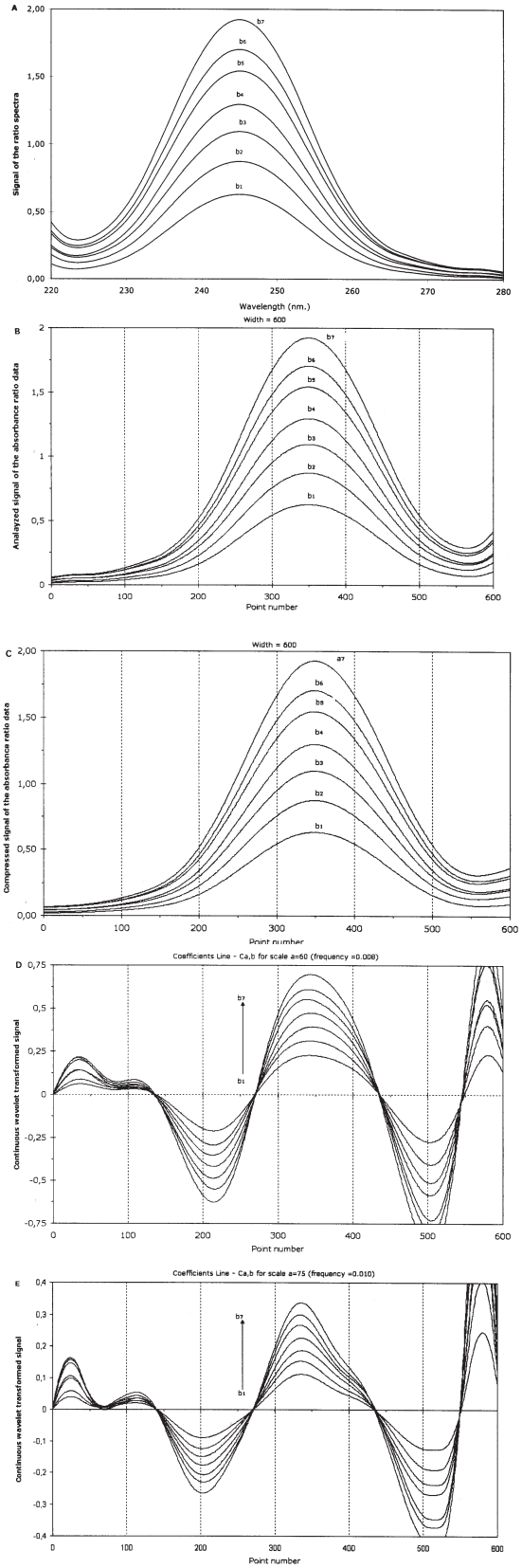


Figure 5. Ratio spectra (A), analyzed spectra (B), compressed spectra (C), gaus4 CWT spectra (D), and coif2 CWT spectra (E) of: (b1) 12 μg/mL, (b2) 16 μg/mL, (b3) 20 μg/mL, (b4) 24 μg/mL, (b5) 28 μg/mL, (b6) 32 μg/mL, (b7) 36 μg/mL BE using 20 μg/mL HCT as a divisor.

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Table 1. Linear regression analysis and its statistical results

Method	Working point	Equation S = m x C + n[a]	r[b]	SE(r)[c]	S(m)[d]	SE(n)[e]	LOD, mg/mL[f]	LOQ , mg/mL[g]
db10 level=6+Gaus4 (a=60)	557 max	$S = 7.3318 \times C_{HCT} - 4.6943$	0.9990	1.7634	0.1666	2.7481	0.86	2.85
	620 min	$S = -12.0510 \times C_{HCT} + 9.3864$	0.9990	2.7740	4.3230	0.2621	0.93	3.08
	218 min	$S = -0.0168 \times C_{BE} - 0.0141$	0.9994	0.0056	0.0003	0.0066	1.78	5.94
	352 max	$S = 0.0190 \times C_{BE} + 0.0045$	0.9991	0.0077	0.0004	0.0092	1.26	4.20
db10 level=6+Coif2 (a=75)	569 max	$S = 7.3318 \times C_{HCT} - 4.6943$	0.9990	1.2056	0.1139	1.8788	0.88	2.92
	622 min	$S = -12.0510 \times C_{HCT} + 9.3864$	0.9990	2.5348	0.2395	3.9502	1.13	3.77
	216 min	$S = -0.0068 \times C_{BE} - 0.0037$	0.9996	0.0019	0.0001	0.0023	1.31	4.38
	349 max	$S = 0.0087 \times C_{BE} + 0.0033$	0.9996	0.0024	0.0001	0.0029	1.72	5.72

[a] S = CWT signal, m = slope of linear regression line, n = intercept of linear regression line, C = concentration (μg/mL).
[b] r = Correlation coefficient.
[c] SE (r) = Standard error of correlation coefficient.
[d] SE (m) = Standard error of slope.
[e] SE (n) = Standard error of intercept.
[f] LOD = Limit of detection.
[g] LOQ = Limit of quantitation.

Table 2. Recovery results obtained for HCT and BE in their binary mixture by 2 wavelet transforms

Binary mixture, mg/mL		Recoveries, % HCT				Recoveries, % BE			
		db10 l=6 + gaus4 a=60		db10 l=6 + coif2 a=75		db10 l=6 + gaus4 a=60		db10 l=6 + coif2 a=75	
HCT	BE	557 max	620 min	569 max	622 min	218 min	352 max	216 min	349 max
20	12	98.9	99.1	99.0	99.2	94.8	96.6	100.6	94.4
20	16	98.1	98.2	98.0	98.2	104.7	107.1	104.4	102.3
20	20	98.3	98.7	98.4	98.7	100.8	99.9	99.3	97.6
20	24	98.9	99.1	98.9	99.1	100.5	103.0	99.1	99.9
20	28	99.5	99.7	99.5	99.7	98	102.7	97.3	98.8
20	32	102.4	102.9	102.5	103.0	99.6	100.3	99.1	99.4
20	36	98.9	99.1	100.0	102.5	100.5	98.7	98.8	100.0
10	16	103.8	103.8	103.9	103.9	101.6	98.5	99.0	105.6
12	16	103.2	103.4	103.3	103.5	99.4	95.4	95.6	102.3
14	16	102.0	102.1	102.1	102.1	97.7	96.8	101.1	99.5
16	16	104.1	105.2	104.4	105.4	94.2	96.9	97.4	96.6
18	16	100.6	100.7	100.6	100.8	95.7	94.8	101.5	100.0
20	16	105.0	105.4	105.1	105.4	105.3	102.7	103.2	106.9
22	16	102.4	102.5	102.4	102.5	98.5	96.4	97.1	101.1
Mean		101.2	101.4	101.3	101.7	99.4	99.3	99.5	100.3
RSD[a]		2.35	2.45	2.34	2.40	3.30	3.56	2.43	3.27

[a] RSD = Relative standard deviation.

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Table 3. Recovery results by applying the standard addition technique to 2 pharmaceutical formulations using CWT methods

	Compress db10 level=6 + gaus4 a=60				Compress db10 level=6 + coif2 a=75			
	Recovery, % HCT							
	557 max		620 min		569 max		622 min	
	I	II	I	II	I	II	I	II
Mean	100.8	101.5	101.1	102.4	101.4	99.7	101.8	103
RSD[a]	2.2	1.95	2.54	1.87	2.05	2.45	1.84	2.26
	Recovery, % BE							
	218 min		352 max		216 min		349 max	
Mean	99.5	100.2	98.2	99.4	98.5	99	98.8	101.6
RSD	2.95	2.88	1.54	2.09	3.1	2.75	1.95	2.91

[a] RSD = Relative standard deviation.

were found to be the optimal choices for our investigated problem.

The application of gaus4 and coif2 CWT to the compressed signals of HCT and BE gives us the coincident amplitudes of the CWT signals in the wavelet domain.

The concentration of HCT using gaus4 and coif2 CWT was proportional to the transformed amplitudes of the maxima at 557 and 569 points and the minima at 620 and 622 points as shown in Figure 4D and 4E, respectively. On the other hand, the concentration of BE was proportional to the transformed amplitudes of the minima at 218 and 216 points and the maxima at 352 and 349 points as shown in Figure 5D and 5E, respectively.

Construction of Calibration Graphs

To determine HCT in its binary mixture with BE by using gaus4 (I) and coif2 (II) CWT, 4 calibration graphs were obtained by measuring the CWT amplitudes of the maxima at 557 (I) and 569 (II) points and of the minima at 620 (I) and 622 (II) points (*see* Figure 4D and 4E). A similar procedure was applied for the determination of BE, and 4 calibration graphs were drawn by measuring the CWT amplitudes of the minima at 218 (I) and 216 (II) and of the maxima at 352 (I) and 349 (II) (*see* Figure 5D and 5E).

The equations of calibration graphs and their statistical results are presented in Table 1. The limit of detection (LOD; signal-to-noise (S/N) ratio is 3:1) and the limit of quantitation (LOQ; S/N ratio is 10:1) were calculated by using the data obtained from 10 replicates for standard solutions of 25 μg/mL for HCT and 16 μg/mL for BE (*see* Table 1).

The calibration range, according to each compound concentration existing in the pharmaceutical commercial products, was designated in the practical range to give accurate, precise, and linear response. The correlation coefficients of calibration curves were found to be higher than 0.999 for all selected 0 maxima and minima points and 2 proposed wavelet methods.

Table 4. Commercial tablet results by applying two wavelet transforms using db10 at level 6

	Compress db10 level=6 + gaus4 a=60				Compress db10 level=6 + coif2 a=75			
	HCT, mg/tablet							
	557 max		620 min		569 max		622 min	
	I	II	I	II	I	II	I	II
Mean	6.34	12.68	6.33	12.63	6.28	12.62	6.52	13.10
SD[a]	0.01	0.26	0.10	0.13	0.07	0.14	0.14	0.47
	BE, mg/tablet							
	218 min		352 max		216 min		349 max	
Mean	5.22	10.41	4.94	9.93	4.91	10.41	4.94	9.99
SD[a]	0.17	0.36	0.10	0.18	0.24	0.36	0.10	0.07

[a] SD = Standard deviation.

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Validation of the Applied Wavelet Method

Two continuous wavelet methods were tested by analyzing different mixtures of the studied compounds at various concentrations within the linearity range. Satisfactory results were obtained (Table 2), indicating the high selectivity of these approaches for determination of 2 compounds in their binary mixtures.

We observed that the obtained results gave good accuracy of the investigated wavelet families, and the mixture results indicated good agreement with literature methods (15–18). Using a column liquid chromatography (LC) method (18), mean percentage recoveries of 99.38 for BE and 99.20 for HCT in the synthetic mixtures were reported. In our case, we found a range of 99.4–100.3 for BE and 100.2–100.7 for HCT. These results indicate that the proposed method is comparable with the LC method.

On the other hand, the accuracy and precision of the wavelet methods were confirmed by applying a standard addition technique in which known amounts of the studied drugs were added to a known concentration of the commercial tablet products. The recovery and its standard deviation results for 5 replicates are given in Table 3. In application of the reported LC method (18) for the standard addition technique, the mean percentage recoveries were 100.06 for BE and 99.29 for HCT. The results obtained by the proposed method for standard addition technique are summarized in Table 3. These results indicate that there was no interference from the excipients used in the formulation of the tablets. It was observed that the results of proposed and reported LC method gave good agreement for the analysis of the 2 compounds.

Analysis of Commercial Tablet Formulations

Two CWT methods were applied to the simultaneous determination of HCT and BE in 2 commercial tablet formulations. Eight replicate determinations for both commercial products were made. Satisfactory results were obtained for the 2 drugs in good agreement with the label claims (*see* Table 4). The results of the proposed wavelet methods for 2 commercial tablet formulations were statistically compared with each other as well as with those of reported literature methods (15–18).

Conclusions

This study offers an application of CWT, based on a ratio procedure of UV-absorption spectra, to the simultaneous analysis of binary mixtures of compounds that have overlapping spectra. For each active compound in a binary mixture, without searching for the critical point for the separated peaks, the maximum or minimum amplitude of the separated peaks can be measured in order to obtain the calibration graphs. This can be considered as an advantage of this approach over derivative spectrophotometry for the quantitative anaysis of binary mixtures. In this study, this approach was proposed for the simultaneous determination of BE and HCT in samples. Although the absorption spectra of BE and HCT overlap in the 215–355 nm wavelength range, the CWT approach provides successful results for the quantitative determination of HCT and BE in binary mixtures and 2 commercial tablet formulations. It was observed that the quantitative determination results obtained in the proposed methods are comparable with LC (16–18). In addition, the method described in this paper does not require any chemical pretreatment, such as a prior separation step that is required in LC. The results also showed that the developed method is more reliable than other spectrophotometric methods (15–17). Finally, the approach can be easily applied to routine analysis, quality control of binary mixtures, and commercial products containing the 2 analyzed drugs.

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