DRUGS, COSMETICS, FORENSIC SCIENCES

Multicomponent Quantitative Resolution of Binary Mixtures by Using Continuous Wavelet Transform

ERDAL DINÇ
University of Ankara, Faculty of Pharmacy, Department of Analytical Chemistry, 06100, Tandoğan, Ankara, Turkey
DUMITRU BALEANU[1]
University of Çankaya, Faculty of Arts and Sciences, Department of Mathematics and Computer Sciences, 06530, Ankara, Turkey

Continuous 1-dimensional wavelet transform (WT) was applied to the quantitative analysis of a vitamin combination of thiamine hydrochloride (THI) and pyridoxine hydrochloride (PYR) with strongly overlapping signals. Absorbance data from the UV-Vis absorption spectrum of width 1150 were subjected to Gauss1 and Gauss2 WTs. Because of its flexibility, data processing, and its high signal amplitude, the continuous WT method is a powerful tool for analysis of multicomponent mixtures. By measuring the amplitude signals corresponding to the selected zero-crossing points of the transformed signal, we obtained the calibration curve. The validation of the calibration graphs was confirmed with different mixtures of THI and PYR at various concentration ratios. A brief explanation of the continuous wavelet method is given. MATLAB 6.5 software was used to perform the calculations. The results of our study were compared with those obtained by spectroscopic, chemometric, and liquid chromatographic methods, and good agreement was found.

The powerful wavelet transform (WT; 1–4) has been applied in many areas, e.g., signal processing (5), de-noising (6), spectral quantitative analysis (7), analysis of electrochemical noise data (8), photoacoustic signal processing (9), and resolution of simulated overlapping spectra (10). Mallat and Hwang (11) developed a fast implementation method for the discrete wavelet method and, as a result, they made the wavelet method an effective tool for processing chemical data. The basic idea of continuous wavelet transform (CWT) is to represent any arbitrary function as a superposition of wavelets or "small waves." In early work, CWT and multiresolution analysis (MRA) were applied to remove noise and irrelevant information in absorption spectra.

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Corresponding author's e-mail: dinc@pharmacy.ankara.edu.tr.
[1] Present address: Institute of Space Sciences, National Institute for Laser, Plasma and Radiation Physics, Magurele-Bucharest, PO Box MG-23, R 76911, Romania.

In the spectrophotometric studies, derivative spectrophotometry and ratio-spectra derivative spectrophotometry were the basic tools for quantitative resolution of mixtures containing ≥2 compounds. Unfortunately, in some cases these methods have a great disadvantage: the higher-derivative process reduces the peak amplitude, the process of finding zero-crossing points becomes difficult, and the sensitivity of the method decreases. The ratio-spectra derivative method gives, in some cases, an infinite value for the ratio spectra.

Thiamine hydrochloride (THI) and pyridoxine hydrochloride (PYR) in mixtures have been determined by spectrophotometric (12), ratio-spectra derivative (13), liquid chromatographic (LC; 13), and chemometric methods (14). These methods have various disadvantages for the determinations due to the separation procedure in the LC method, the diminished peak amplitude in the higher-derivative method, and the complex calculations in the chemometric method. In this context, the wavelet technique (1–4) is a promising tool for eliminating the above drawbacks. The simultaneous performance of data reduction and de-noising for signal analysis (4) is one of the main advantages of WT.

The aim of this study was to apply Gauss continuous wavelet transform (GCWT) to the simultaneous determination of THI and PYR in a mixture.

The first step was to obtain significant peak amplitude for the transformed signal. To reach this objective, we used 2 different Gauss mother wavelets, GCWT1 and GCWT2. In the second step, the transformed signal was subjected to a zero-crossing technique.

METHOD

Wavelet Transform

Wavelets are an extension of Fourier analysis. The goal is to turn the information in a signal into numbers, which can be stored, transmitted, analyzed, or used to reconstruct the original signal. The coefficients tell in what way the analyzing function needs to be modified in order to reconstruct a signal. We can construct the signal by putting together wavelets of different sizes at different positions. The signal and the analyzing function are multiplied together, and the integral of the product is calculated. Wavelets adapt immediately to the dif-

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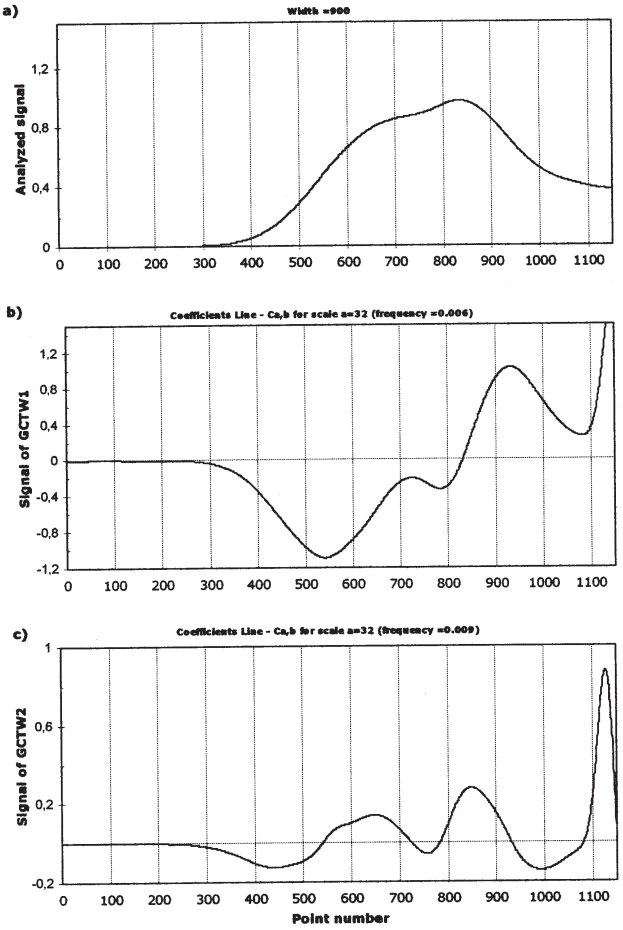


Figure 1. (a) Analyzed signal, (b) GCWT1 signal, and (c) GCWT2 signal corresponding to THI at 24 μg/mL.

ferent components of a signal. A small window is used to look at brief, high-frequency components, and a large window, to look at long-lived, low-frequency components.

Continuous Wavelet Transform

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

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

where a denotes 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-varying signal (λ), WTs contain the computation of coefficients obtained from the inner products of the signal and a family of wavelets. CWT of $f(\lambda)$ is defined as follows:

$$\mathrm{CWT}\,\{\mathrm{f}\,(\lambda);\; a,\; b\} \;=\; \int_{-\infty}^{\infty} f(\lambda)\Psi^{*}_{a,\,b}(\lambda)\,\mathrm{d}\lambda \;=\; \left\langle \mathrm{f}(\lambda), \Psi_{a,\,b} \right\rangle \qquad (2)$$

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

The wavelet Ψ is invertible if it satisfies the admissibility condition

$$\int_{-\infty}^{\infty} \frac{\left|\hat{\Psi}(\omega)\right|2}{\omega}\,\mathrm{d}\omega \;<\; \infty \qquad (3)$$

In this case the initial signal is reobtained from $\Psi_{\mathrm{a},\,b}$:

$$\mathrm{f}(\lambda) = \frac{1}{\mathrm{C}} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \mathrm{CWT}\,(a,\; b)\,\Psi_{a,b}\,\frac{\mathrm{da\;db}}{\mathrm{a}^{2}} \qquad (4)$$

where C is given by

$$\mathrm{C} = \int_{0}^{\infty} \frac{\hat{\Psi}(\omega)\hat{\Psi}(\omega)}{\omega}\mathrm{d}\omega \qquad (5)$$

and $\hat{\Psi}$ denotes the Fourier transform of Ψ.

By definition, a wavelet has an integral of zero, half the area it surrounds is positive, and the other half is negative. If we multiply the wavelet by a constant, it changes the positive and the negative parts equally, and the integral remains zero.

In this paper, we will focus on the Gaussian wavelets, namely, $f_a(\lambda) = \frac{1}{2}(\pi\alpha)^{-\frac{1}{2}} e^{-\frac{\lambda^2}{4a}}$ and its derivatives of order 1 (GCWT1) and order 2 (GCWT2).

Experimental

Instruments

(**a**) *US-Vis spectrophotometer.*—To record the absorption spectra, a Shimadzu UV-160 double-beam UV-Vis spectrophotometer having a fixed slit width (2 nm), connected to a computer loaded with Shimadzu UVPC software and a Hewlett-Packard DeskJet 600 printer, was used.

(**b**) *Software.*—Excel and MATLAB 6.5 software performed the treatment and transformation of the experimental data.

Commercial Vitamin Tablet

A commercial vitamin tablet (Benexol® film-coated tablet, Batch No. 10169; Roche Pharmaceutical Ind., (Instanbul, Turkey) with a label claim of 250 mg PYR and 250 mg THI per tablet was used. Roche Pharm. Ind. kindly donated the vitamin compounds.

Standard Solutions

Stock solutions of PYR and THI, each at 100 mg/mL, were prepared in 0.1M HCl. The standard solutions, in 25 mL volumetric flasks containing both vitamins and their synthetic mixtures at 8–40 μg/mL, were obtained from the stock solutions.

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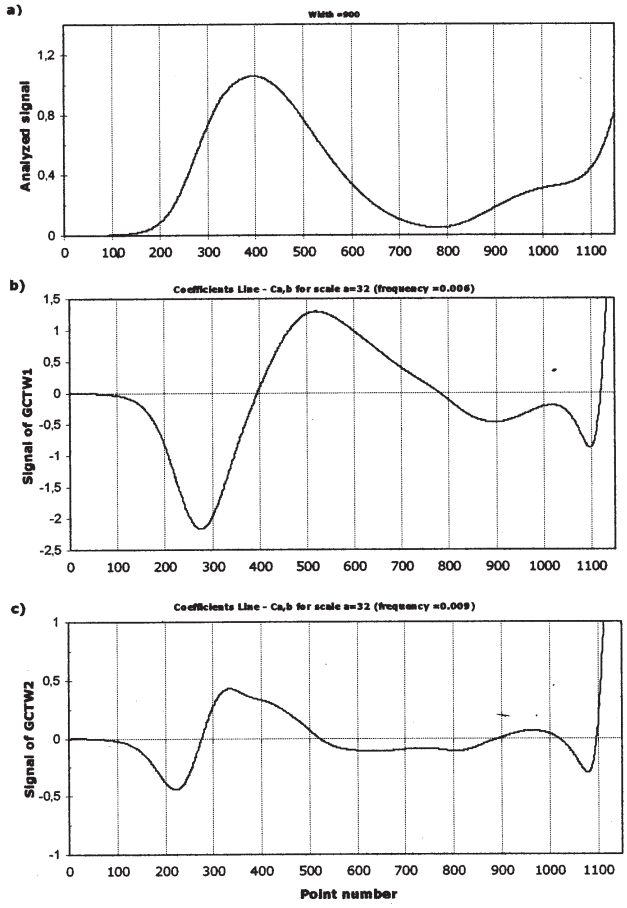


Figure 2. (a) Analyzed signal, (b) GCWT1 signal, and (c) GCWT2 signal corresponding to PYR at 24 μg/mL.

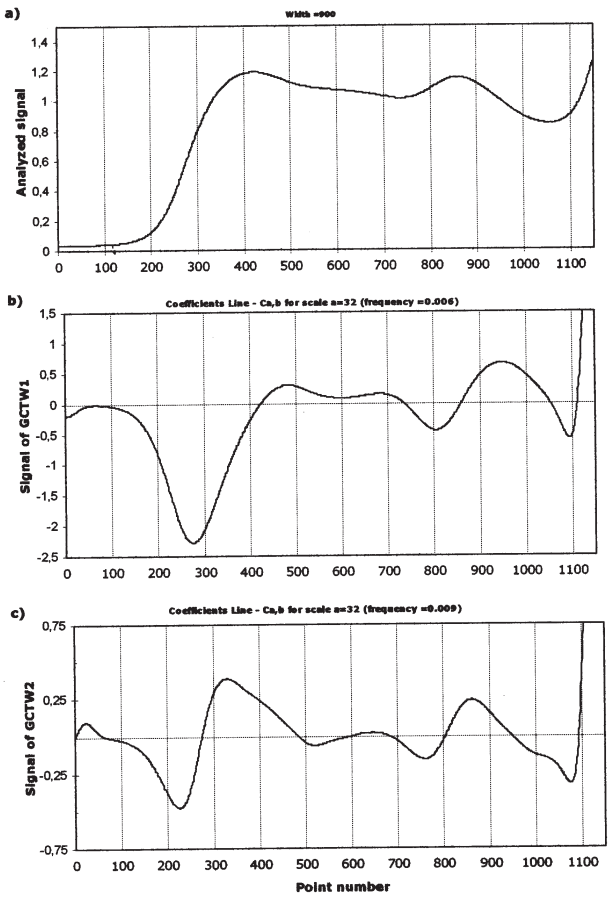


Figure 3. (a) Analyzed signal, (b) GCWT1 signal, and (c) GCWT2 signal corresponding to a mixture of THI and PYR, each at 24 μg/mL.

The recorded absorption spectra in the range of 215–330 nm represent the analyzed vector signal.

Results and Discussion

Experimental Conditions and Optimization

The spectra of PYR and THI, in the concentration range of 8–40 μg/mL, were recorded and transferred to the MATLAB 6.5 software. To apply the CWT approach, our original spectra consisting of 1151 points in the spectral range of 215.0–330.0 nm were adjusted to 1150 points during the transfer to MATLAB 6.5. The number of points used is important. If the working range contains many points, we observed that the intensity of the transformed signal increased. In other words, we optimized the relationship between the number of points and the amplitude of the transformed signal.

The wavelength (λ) plays the role of parameter *t* in CWT analysis, and the wavelength number in our case runs from 1 to 1150. The main problem is to choose an appropriate CWT such that the calibration process will give a correct prediction of the concentrations of the compounds in the samples. The next step is to analyze the transferred signal by using GCWT1 and GCWT2 for several values of scale factor *a*. The transformed signals corresponding to the scale factor $a = 32$ and frequencies 0.006 for GCWT1 and 0.009 for GCWT2 (Figures 1–3) were retained. In these figures the coefficients $C_{a,b}$ are given by Equation 2.

Optimized parameters provide a significant advantage in the investigation of the 2 transformed signals. In addition, the amplitude of the first transformed compound corresponds to the zero-crossing point of the second compound. Taking into account this important result, we decided to apply a zero-crossing technique on the transformed signal.

CWT and Beer-Lambert Law

We consider a binary mixture containing 2 analytes (*X* and *Y*). If the Beer-Lambert law is valid for 2 analytes at all points in the working range, the analyzed signal of the binary mixture at the point *i* is given by

$$S_{mix},\ i = \alpha_{x,\,i}\, C_X + \beta_{Y,i} C_Y \qquad (6)$$

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Table 1. Statistics of the linear regression analysis

Method	Range, μg/mL	Equation[a]	r[b]	S_r[c]	S_m[d]	S_b[e]	LOD, μg/mL[f]	LOQ, μg/mL[g]
GCWT1	8–40	$^1S_{396} = -0.00590 - 0.01384\ C_{THI}$	–0.9998	0.3417	0.0961	0.1558	0.81	2.70
		$^1S_{779} = 0.00257 - 0.01405\ C_{THI}$	–0.9998	0.3443	0.0968	0.1570	0.94	3.1
		$^1S_{831} = -0.00092 - 0.01225\ C_{PYR}$	–0.9999	0.3215	0.0904	0.1466	0.87	2.9
GCWT2	8–40	$^2S_{896} = 0.00360 + 0.00665\ C_{THI}$	0.9997	0.0031	0.0008	0.0014	0.75	2.51
		$^2S_{1017} = -0.00155 - 0.01539\ C_{THI}$	–0.9997	0.2131	0.0599	0.0972	0.79	2.63
		$^2S_{724} = -0.00313 - 0.00352\ C_{PYR}$	–0.9991	0.1723	0.0484	0.0785	1.05	3.52
		$^2S_{784} = -0.00289 - 0.03342\ C_{PYR}$	–0.9995	0.3981	0.1119	0.1815	0.84	2.80

[a] C_{THI} = THI concentration (μg/mL); C_{PYR} = PYR concentration (μg/mL); and S = transformed signal.
[b] r = Regression coefficient.
[c] S_r = Standard deviation of regression.
[d] S_m = Standard deviation of the slope.
[e] S_b = Standard deviation of the intercept.
[f] LOD = Limit of detection.
[g] LOQ = Limit of quantitation.

Here $S_{mix,i}$ represents the analyzed signal of the binary mixture at the point *i*, and $\alpha_{X,i}$ and $\beta_{Y,i}$ denote the constants of X and Y, and C_X and C_Y are the concentrations of X and Y, respectively. We apply CWT on Equation 6 and we obtain

$$\text{CWT } S_{mix,\,i} = \text{CWT } \alpha_{X,i} C_X + \text{CWT} \beta_{Y,i} C_Y \quad (7)$$

We say that the transformed signal admits a zero-crossing point if its amplitude is zero.

Equation 7 is valid locally for a given point *i* and, in this study, we retained only the points for which this law is valid; in other words, we kept only the optimal zero-crossing points. The main observation is that, at a given point *i*, if the concentration increased, the amplitude of the transformed signal increased. If the transformed amplitude (CWT $S_{Y,i}$) of C_Y corresponds to a zero-crossing point, Equation 7 becomes the following:

$$\text{CWT } S_{mix,i} = \text{CWT } \alpha_{X,i}\ C_X \quad (8)$$

Equation 8 indicates that the "transformed spectrum" of the binary mixture depends only on C_X. The amplitude value (CWT $S_{X,i}$) of X is plotted versus C_X at the point corresponding to zero crossing of the transformed signal of Y at the same point (CWT $S_{Y,i}$ is equal to zero). A linear regression line of X is obtained by plotting the transformed amplitude values (CWT $S_{X,i}$) versus the concentration of X at *i* corresponding to the zero-crossing points of the transformed amplitude corresponding to Y. The analyte Y is subjected to a similar procedure.

Calibration Process

Calibration graphs based on GCWT1 and GCWT2 were constructed by measuring the transformed signal at 396 ($^1\mathbf{S}_{396}$) and 779 ($^1\mathbf{S}_{779}$) for THI (corresponding to a zero-crossing point of PYR) and at 831 ($^1\mathbf{S}_{831}$) for PYR (corresponding to a zero-crossing point of THI) and by measuring the signal amplitude at 896 ($^2\mathbf{S}_{896}$) and 1017 ($^2\mathbf{S}_{1017}$) for THI (corresponding to a zero-crossing point of PYR) and at 724 ($^2\mathbf{S}_{724}$) and 784 ($^2\mathbf{S}_{784}$) for PYR (corresponding to a zero-crossing point of THI), respectively. The amplitude values of the transformed signals were drawn as a graph versus concentrations of THI and PYR, and a straight line was obtained for both vitamins at the above-indicated points. By using the calibration equations (Table 1), THI and PYR were determined in the sample. In all calibration equations, high values for the regression coefficients (r) were obtained. The limit of detection (LOD; signal-to-noise ratio of 3:1) and the limit of quantitation (LOQ; signal-to-noise ratio of 10:1) were calculated by using the data obtained from 10 replicates for standard solutions of THI and PYR at 25 μg/mL. Table 1 contains the values for all parameters of the linear regression analysis.

Validation Procedure

In order to validate the above calibration graphs, we prepared mixtures of different compositions in our laboratory. We applied the above signal-analysis procedure to synthetic mixtures (Figure 3) for the determination of the concentrations of both compounds, and we concluded that GCWT1 and

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Table 2. Recovery of THI and PYR from mixtures by GCWT1 and GCWT2

			Recovery, %						
			GCWT1			GCWT2			
	Mixture		THI		PYR	THI		PYR	
No.	THI, μg/mL	PYR, μg/mL	$^1S_{396}$	$^1S_{779}$	$^1S_{831}$	$^2S_{896}$	$^2S_{1017}$	$^2S_{724}$	$^2S_{784}$
1	24	8	102.4	101.3	104.0	99.2	99.5	96.9	102.7
2	24	16	101.2	99.8	100.5	98.2	98.8	99.2	101.2
3	24	24	104.0	100.5	98.8	99.9	98.6	100.1	99.9
4	24	32	105.6	100.8	99.6	101.5	100.0	102.2	101.5
5	24	40	104.5	97.1	98.4	99.9	99.1	101.6	100.4
6	8	24	104.6	106.2	100.3	97.3	99.9	102.4	101.4
7	16	24	105.1	100.6	99.4	99.2	98.7	100.7	100.5
8	24	24	102.4	98.8	100.4	97.6	99.2	100.9	100.8
9	32	24	103.2	101.7	99.1	100.1	104.3	98.6	99.0
10	40	24	102.4	101.2	96.6	99.7	101.1	94.0	97.1
Mean			103.5	100.8	99.7	99.2	99.9	99.7	100.5
RSD, %[a]			1.39	2.34	1.91	1.28	1.73	2.62	1.54

[a] RSD = Relative standard deviation.

GCTW2 gave satisfactory results (Table 2). The validation results indicated that the calculated calibrations at the selected points are useful for determining the concentrations of the 2 compounds in the prepared mixtures. During the analysis procedure, interference and systematical errors were not observed.

Tablet Analysis

Ten tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in 0.1M HCl in a 100 mL volumetric flask by sonication. The solution was filtered into a 100 mL volumetric flask through Whatman No. 42 filter paper, and the filtrate was diluted to volume with the same solvent. GCWT1 and GCWT2 were then applied to tablet analysis. The experimental results of tablet analysis are presented in Table 3. The excipients in the tablets give a constant signal, which was eliminated in the working range of the transformed spectrum. This is a consequence of the WT as well as a matter of choosing the zero-crossing points. In other words, our method can be used

Table 3. Results (mg/tablet) obtained by using GCWT1 and GCWT2 for the analysis of commercial tablets

	GCWT1			GCWT2			
	THI		PYR	THI		PYR	
Parameter	$^1S_{396}$	$^1S_{779}$	$^1S_{831}$	$^2S_{896}$	$^2S_{1017}$	$^2S_{724}$	$^2S_{784}$
Mean[a]	250.4	252.5	254.2	253.5	251.9	251.2	253.4
SD[b]	2.62	3.03	4.15	4.61	3.14	4.07	3.48
RSD, %[c]	1.05	1.17	1.62	1.82	1.23	1.59	1.36
SE[d]	1.51	1.75	2.39	2.66	1.81	2.35	2.01
CL (P = 0.05)[e]	3.05	3.53	4.82	5.37	3.66	4.73	4.05

[a] n = 10.
[b] SD = Standard deviation.
[c] RSD = Relative standard deviation.
[d] SE = Standard error.
[e] CL = Confidence limit.

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Table 4. Results (mg/tablet) obtained for the analysis of commercial tablets by other methods reported in the literature

Method	THI mean ± SD[a]	PYR mean ± SD
Absorbance ratio	248.7 ± 4.3	248.8 ± 4.0
First-derivative ratio spectra	249.6 ± 0.8	248.8 ± 0.8
Liquid chromatography	250.0 ± 0.9	249.8 ± 0.9
chemometry[b]		
PCA	248.9 ± 0.8	248.8 ± 0.7
CLS	248.6 ± 0.8	248.8 ± 1.7
ILS	248.7 ± 1.1	248.8 ± 1.1

[a] SD = Standard deviation.

[b] PCA = Principal least squares; CLS = classical least squares; and ILS = inverse least squares.

in the presence of interference with the transformed spectra of both active compounds and in the presence of the excipients from tablets.

Methods based on absorbance ratio (12), first derivative of the ratio spectra (13), LC (12), and chemometrics (13) had already been applied to the same commercial vitamin combination, and these experimental results are summarized in Table 4.

Conclusions

WT is a promising method for future investigation of the analysis of overlapping signals and the simultaneous analysis of mixtures.

In this paper, we proposed a method based on CWT to analyze the signal of THI and PYR in a mixture. We observed that, after 1-dimensional GCTW1 and GCWT2 were performed on the signal, the amplitude of the transformed signal increased with respect to results obtained by the conventional derivative methods. The transformed signal and the concentration have a very good linear correlation for the measured amplitudes corresponding to the zero-crossing points in the working width. The results obtained are reliable in comparison with those given by spectroscopic (12, 13) and chemometric (14) methods and comparable with those delivered by LC (13).

Because we have many families of wavelets, we can choose an appropriate one for quantitative analysis of the mixture systems. Each CWT family has some parameters, which can be optimized to obtain the maximum resolution. The investigated method does not require any other complex calibration technique, and it is a power tool for the resolution of binary-mixture systems. The method is easy to apply, fast, and very inexpensive.

The proposed method is suitable for application to quality control and the routine analysis of mixtures and commercial products.

The software for CWT can be successfully used with UV-Vis spectrophotometers and other analytical instruments.

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