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A zero-crossing technique for the multidetermination of thiamine HCl and pyridoxine HCl in their mixture by using one-dimensional wavelet transform

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Abstract

A new zero-crossing technique based on one-dimensional wavelet transform (WT) was developed and applied on a commercial vitamin product and binary mixtures containing thiamine HCl and pyridoxine HCl in the presence of the interference of the analysed signals. We selected from the data of the UV–Vis absorption spectra a signal consisting of 1150 points corresponding to the concentration range 8–32 mg/ml for both vitamins and we subjected it to one-dimensional continuous WT Mexican (MEXICAN) and Meyer (MEYER). Since the peaks of the transformed signals were bigger than original ones a zero crossing technique was applied to obtain the regression equations. The validity of Beer–Lambert law was assumed for the transformed signals. An appropriate scale setting was choosing to obtain an alternative calibration for each method. The basic concepts about wavelet method were briefly explained and MATLAB 6.5 software was used for one-dimensional wavelet analysis. The obtained results were successfully compared among each other and with those obtained by other literature methods. The developed method is rapid, easy to apply, not expensive and suitable for analysing of the overlapping signals of compounds in their mixtures without any chemical pre-treatment.

Keywords: Continuous one-dimensional Meyer and Mexican wavelet transforms; Multicomponent determination; Thiamine HCl; Pyridoxine HCl

1. Introduction

The multicomponent determination of drugs in the pharmaceuticals containing two or more active compounds is one of the main problems of the drug analysis.

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To solve this problem several analytical methods were developed but still the resolving of the multi-mixtures has drawbacks in some cases [1–11].

The normal derivative and its version, ratio spectra derivative methods have been used for the resolving of the mixtures in the spectral studies. Since the higher derivative process reduce the peak amplitude, the process of finding zero-crossing points is very difficult and the sensitivity of the method is decreasing. Particularly, the ratio spectra derivative method leads us, in some cases, to an infinite value of ratio spectra.

For these reasons, new methods should be discovered and applied to the analytical problems. One of those promising methods is wavelet transform (WT) [12–22] rapidly developed during the last decade in various branches, e.g. signal processing [23,24], de-noising [25,26], spectral quantitative analysis [27], analysis of electrochemical noise data [28,29], photoacoustic signal processing [30], resolving simulated overlapped spectra [31] or flow-injection analysis [32]. The basis idea of continuous wavelet transformation (CWT) is to represent any arbitrary function as a superposition of wavelets. A fast implementation method for discrete wavelet method was discovered by Mallat and Hwang [33] and it is an effective tool for processing chemical data.

The main aim of this paper is to apply MEXICAN and MEYER WT to the multicomponent determination of thiamine HCl and pyridoxine HCl in their combination.

2. Wavelet transform

2.1. Continuous wavelet transform

Wavelet or 'small waves' is expressed as a series of functions $\Psi_{a,b}(\lambda)$ possessing forms as:

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

Here a denotes the scale parameter which is a variable used to control the scaling and b represents the translation parameter controlling the translation and R is the domain of real numbers. A mother wavelet $\Psi(\lambda)$ generates the set of functions $\Psi_{a,b}(\lambda)$ by scaling (or dilatation) and shifting (or translation). CWT of $f(\lambda)$ is defined as:

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

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

We say that the wavelet Ψ is invertible if it satisfies the admissibility condition:

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

The original signal is reobtained from $\Psi_{a,\mathrm{b}}$ as:

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

Here C has the following expression:

$$C = \int_0^{\infty} \frac{\hat{\Psi}^*(\omega)\hat{\Psi}(\omega)}{\omega}\ \mathrm{d}\omega, \qquad (5)$$

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

2.2. Mexican and Meyer wavelets

MEXICAN is defined as:

$$\Psi(\lambda) = 2\pi\omega^{-1/2}\left[1 - 2\pi\left(\frac{\lambda}{\omega}\right)^2\right]c^{-\pi(\lambda/\omega)^2}, \qquad (6)$$

where ω represents the width parameter (e.g. $\omega = 1/16$).

The Fourier transform of the corresponding Meyer scale function is defined as:

$$\hat{\phi}(\omega) = \begin{cases} 1 & |\omega| \leq \frac{2}{3}\ \pi \\ \cos\left[\frac{\pi}{2}\ v\left(\frac{3}{2\pi}|\omega| - 1\right)\right] & \frac{2}{3}\ \pi \leq |\omega| \leq \frac{4}{3}\ \pi \\ 0 & \text{otherwise.} \end{cases} \qquad (7)$$

where $v(x)$ is an arbitrary C^{∞} function satisfying:

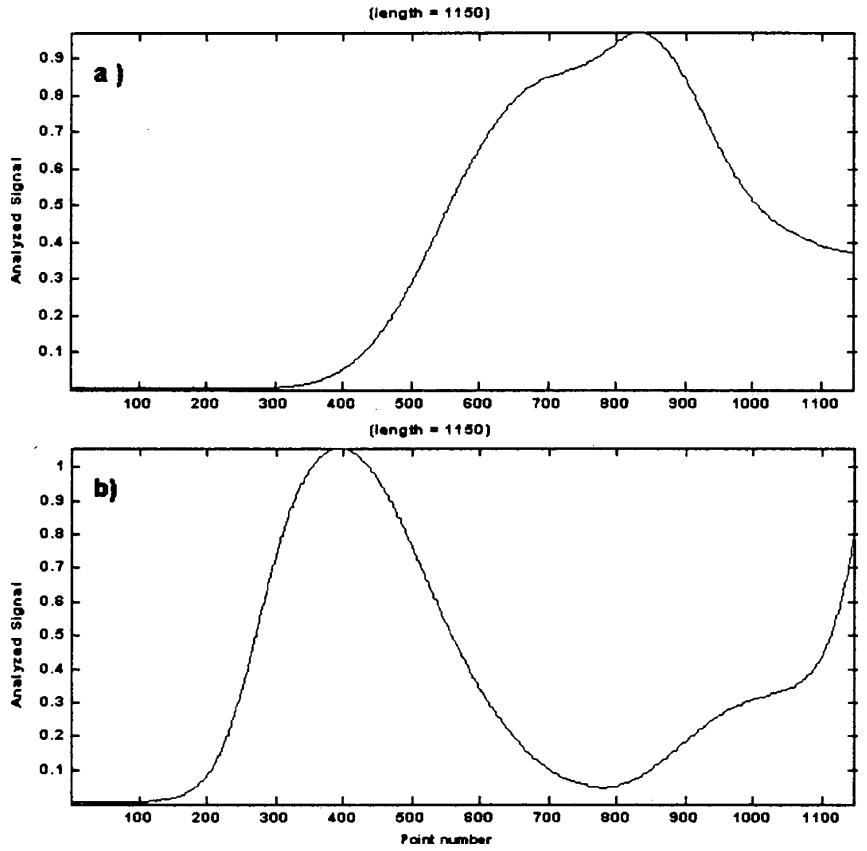

Fig. 1. Analysed signal of (a) 24 μg/ml thiamine HCl and (b) 24 μg/ml pyridoxine HCl in 0.1 HCl.

$$v(x)=\begin{cases}0 & x\le 0\\ 1 & x\ge 1\end{cases}\quad\text{and}\quad v(x)+v(x)+v(1-x)=1\quad(\forall x\in[0,\ 1]) \tag{8}$$

It can be proven that the filter function is:

$$m_0(\omega)=\sum_{k-\infty}^{\infty}\hat{\phi}(2(\omega+2\pi k)) \tag{9}$$

and Meyer wavelet Ψ, which is an orthonormal wavelet, is then defined by:

$$\hat{\Psi}(\omega)=c^{-i(\omega/2)m_0(\omega/2+\pi)\hat{\phi}(\omega/2)} \tag{10}$$

It can be shown that:

$$\hat{\Psi}(\omega)=\begin{cases}e^{-i(\omega/2)\sin[(\pi/2)v(3/2\pi|\omega|-1|)]} & \dfrac{2\pi}{3}\le|\omega|\dfrac{4\pi}{3}\\ c^{-1(\omega/2)\cos[(\pi/2)v(3/4\pi|\omega|-1)]} & \dfrac{4\pi}{3}\le|\omega|\dfrac{8\pi}{3}\\ 0 & \text{otherwise.}\end{cases} \tag{11}$$

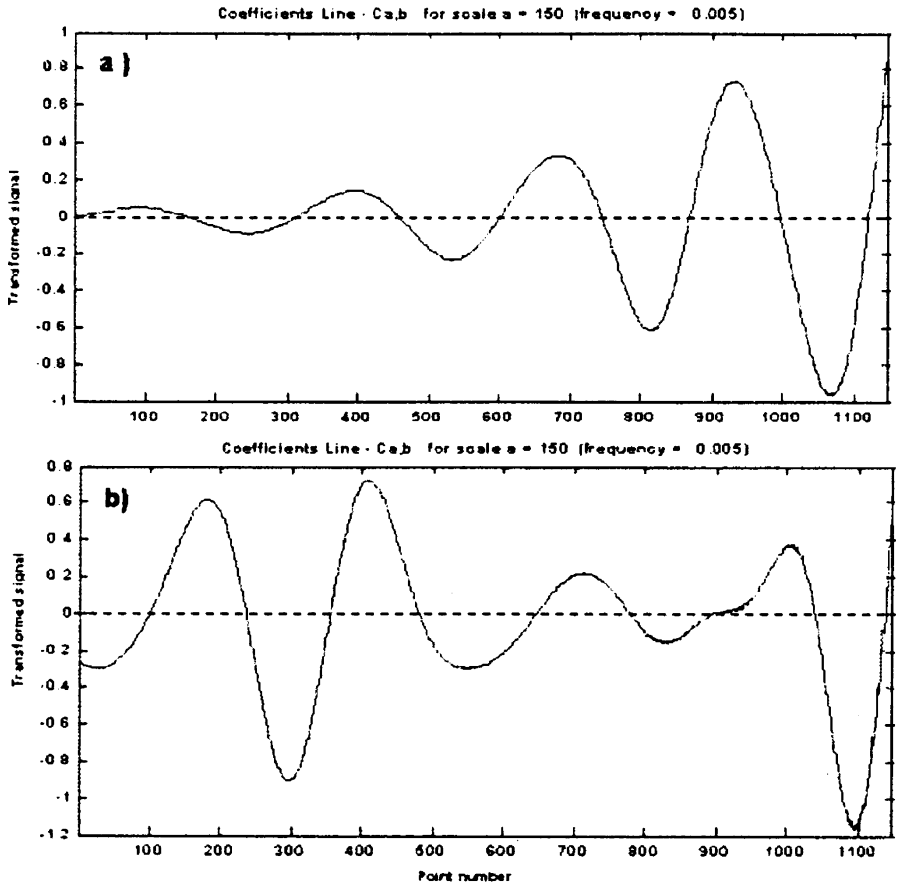

Fig. 2. Transformed signal of (a) 24 μg/ml thiamine HCl and (b) 24 μg/ml pyridoxine HCl by MEYER.

3. Experimental

3.1. Instruments

a) A Shimadzu UV-1600 double beam UV–Vis spectrophotometer connected to a computer having Shimadzu UVPC software and a HP DeskJet 600 printer were used to record the UV–Vis absorption spectra.
b) Calculations and the signal analysis were obtained by using EXCEL and MATLAB 6.5.

3.2. Vitamin formulation

In this paper *a* vitamin tablet (Benexol® film-coated tablet, Roche Pharm. Ind., Turkey, Batch no. 10169) consisting of 250 mg pyridoxine hydrochloride and 250 mg thiamine hydrochloride per tablet was investigated. Roche Pharm. Ind., Turkey kindly donated the active vitamin compounds.

3.3. Standard solutions

Stock solutions of 100 mg/100 ml of thiamine HCl and pyridoxine HCl were prepared by using 0.1 M HCI. The standard solutions in 25-ml volumetric flasks containing 8–40 μg/ml for both vitamins and their synthetic mixtures were obtained from their stock solutions.

The analysed signal subjected to MEXICAN and MEYER wavelet transformations represents the recorded absorption spectra in the range of 215–330 nm.

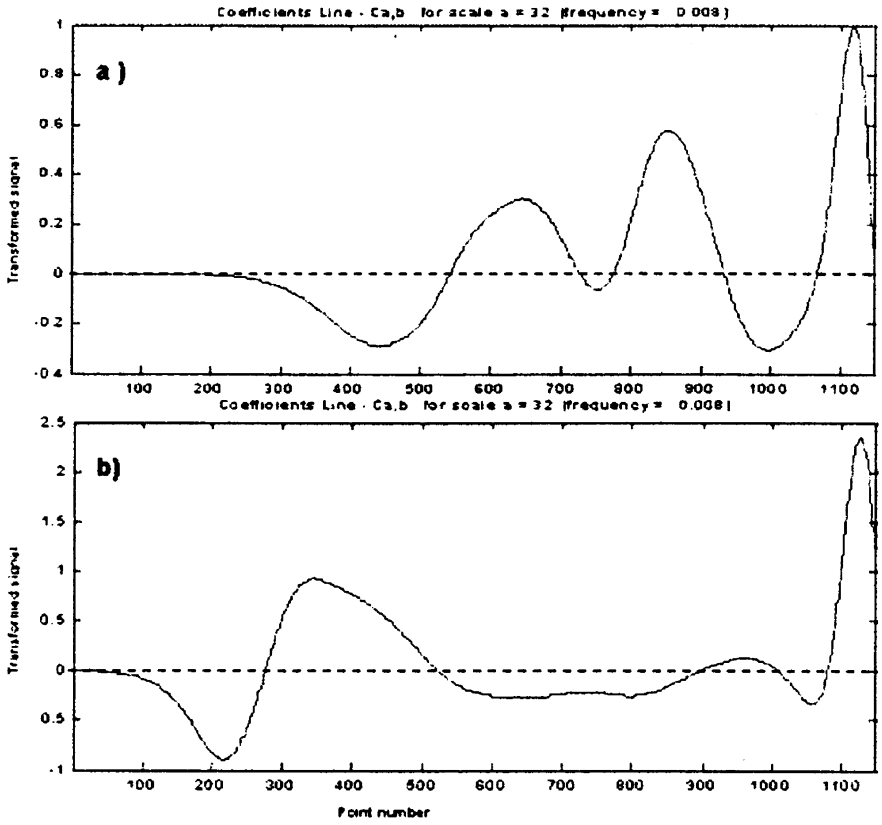

Fig. 3. Transformed signal of (a) 24 μg/ml thiamine HCl and (b) 24 μg/ml pyridoxine HCl by using MEXICAN.

4. Results and discussion

4.1. Experimental conditions

A standard solution containing thiamine HCl and pyridoxine HCl in the concentration range of 8–40 μg/ml was prepared and its spectra were recorded in the range of 215.0–330.0 nm. We selected 1150 points from the original spectra to make possible the wavelet analysis and we transferred it to MATLAB 6.5 software for signal analysis process (see Fig. 1). The wavelength (λ) plays the role of parameter t in CWT analysis, then the coefficients C_{ab} can be plotted versus wavelength number (in this case it runs from 1 to 1150). We investigated different values for scale parameter to obtain the maximum values of peaks corresponding to the transformed signals. The optimal values of the scale factor and frequencies were $a = 150$ and 0.005 for MEYER and $a = 32$ and 0.008 for MEXICAN (see Figs. 2 and 3). A similar calibration concept as in zero-crossing techniques on transformed signals was used. We observed that if we decrease the length of the signal the peak intensity of the transformed signals become smaller and the zero crossing points were diminished (Fig. 4).

4.2. CWT and Beer–Lambert law

We consider a binary mixture containing two analytes (X and Y). If the Beer–Lambert law is valid for two analytes at the whole points in the working range, the analysed signal of the binary mixture at the point i has the form:

$$S_{\mathrm{mix},i} = \alpha_{X,i} C_X + \beta_{Y,i} C_Y \quad (12)$$

Here $S_{\mathrm{mix},i}$ represents the analysed signal of the binary mixture at the point i, $\alpha_{X,i}$, β_{Y,p_i} denote the

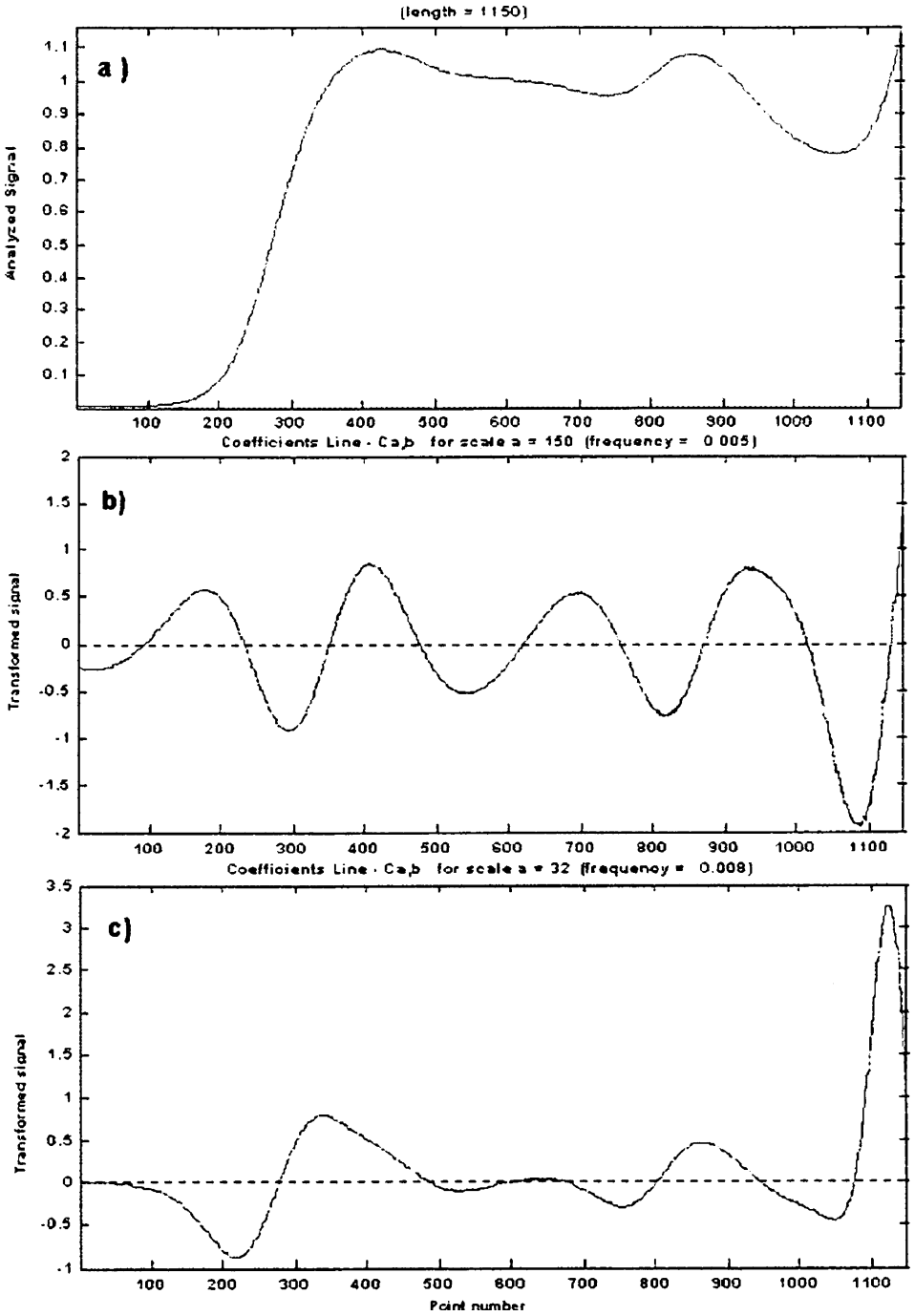

Fig. 4. (a) Analysed signal; (b) MEYER and (c) MEXICAN of the mixture containing 24 μg/ml thiamine HCl and pyridoxine HCl.

Table 1
Calibration graphs data

Methods	Range (μg/ml)	Equation	r	S_r	S_m	S_b	LOD (μg/ml)	LOQ (μg/ml)
MEXICAN	8–40	${}^{mexh}S_{242} = -0.02700 - 0.02848C_{PYR}$	−0.9983	0.4902	0.1378	0.2235	0.68	2.27
		${}^{mexh}S_{726} = -0.00820 - 0.00858C_{PYR}$	−0.9991	0.2690	0.0756	0.1226	0.70	2.33
		${}^{mexh}S_{774} = -0.00600 - 0.00940C_{PYR}$	−0.9994	0.2816	0.07992	0.1284	1.07	3.56
		${}^{mexh}S_{934} = 0.00460 + 0.00370C_{PYR}$	0.9961	0.0079	0.0022	0.0036	1.00	3.32
		${}^{mexh}S_{524} = -0.00100 - 0.00388C_{THI}$	−0.9992	0.1808	0.0508	0.0824	0.82	2.73
		${}^{mexh}S_{897} = 0.00510 + 0.01451C_{PYR}$	0.9999	0.0020	0.0006	0.0009	0.67	2.24
		${}^{mexh}S_{1010} = 0.00050 - 0.01259C_{PYR}$	−0.9996	0.3258	0.0916	0.1486	0.39	1.29
MEYER	8–40	${}^{meyr}S_{160} = 0.02280 + 0.02280C_{PYR}$	−0.9993	0.0081	0.0023	0.0037	0.57	1.89
		${}^{meyr}S_{313} = -0.02269 - 0.03262C_{PYR}$	−0.9994	0.5246	0.1475	0.2392	0.41	1.35
		${}^{meyr}S_{460} = -0.00436 + 0.01072C_{PYR}$	0.9988	0.0073	0.0021	0.0033	0.56	1.87
		${}^{meyr}S_{601} = -0.00112 - 0.00843C_{PYR}$	−0.9996	0.2667	0.0750	0.1216	0.50	1.68
		${}^{meyr}S_{745} = 0.00054 + 0.00647C_{PYR}$	0.9993	0.0043	0.0012	0.0020	0.65	2.18
		${}^{meyr}S_{868} = 0.00307 - 0.00258C_{PYR}$	−0.9971	0.1476	0.0415	0.0673	1.23	4.09
		${}^{meyr}S_{997} = 0.01514 + 0.01368C_{PYR}$	0.9983	0.0098	0.0028	0.0045	0.91	3.04
		${}^{meyr}S_{482} = -0.00384 - 0.00365C_{THI}$	−0.9980	0.1755	0.0494	0.0800	0.29	0.98
		${}^{meyr}S_{646} = -0.00303 + 0.01053C_{THI}$	0.9994	0.0050	0.0014	0.0023	0.62	2.06
		${}^{meyr}S_{779} = 0.00293 - 0.01699C_{THI}$	−0.9998	0.3785	0.1064	0.1726	0.77	2.56
		${}^{meyr}S_{896} = -0.00774 + 0.02031C_{THI}$	0.9998	0.0039	0.0011	0.0018	0.39	1.29

C_{THI}, THA concentration (μg/ml); r, regression coefficient; C_{PYR}, PYR concentration (μg/ml); S_r, standard deviation of regress; ${}^{db}S$ and ${}^{db}S$, transformed signal of MEXICAN and MEYER; S_m, standard deviation of slope; S_b, standard deviation of intercept; LOD, limit of detection; LOQ, limit of quantification.

constants of X and Y, C_X and C_Y are the concentration of X and Y, respectively.

If we apply CWT on the Eq. (12) we obtain:

$$\mathrm{CWT}S_{\mathrm{mix},i} = \mathrm{CWT}\alpha_{X,i}C_X + \mathrm{CWT}\beta_{Y,i}C_Y \quad (13)$$

The Eq. (13) can be considered as the Beer–Lambert law for the transformed signal.

This equation is valid only 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 ($\mathrm{CWT}S_{Y,i}$) of C_Y corresponds to a zero crossing point (in other words if $C_Y = 0$), the Eq. (13) becomes:

$$\mathrm{CWT}S_{\mathrm{mix},i} = \mathrm{CWT}\alpha_{X,i}C_X \quad (14)$$

Eq. (14) indicated that the 'transformed spectrum' of the binary mixture depends only on C_X. The amplitude value ($\mathrm{CWT}S_{X,i}$) of X is plotted versus C_X at the point corresponding to zero crossing of the transformed signal of Y in the same point ($\mathrm{CWT}S_{Y,i}$ is equal to 0).

Linear regression line of X is obtained by plotting the transformed amplitude values ($\mathrm{CWT}S_{X,i}$) 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.

4.3. Regression equations

The calibration graphs of MEYER were obtained by measuring the transformed signal at 160 (${}^{meyr}S_{160}$), 313 (${}^{meyr}S_{313}$), 460 (${}^{meyr}S_{460}$), 601 (${}^{meyr}S_{601}$), 745 (${}^{meyr}S_{745}$), 868 (${}^{meyr}S_{868}$) and 997 (${}^{meyr}S_{997}$) for pyridoxine HCl (corresponding to zero crossing point of thiamine HCl) and at 482 (${}^{meyr}S_{482}$), 646 (${}^{meyr}S_{646}$), 779 (${}^{meyr}S_{779}$) and 896 (${}^{meyr}S_{896}$) for thiamine HCl (corresponding to zero crossing point of pyridoxine HCl). By using a similar procedure, the regression equations for MEXICAN were constructed by measuring the signal amplitude at 242 (${}^{mexh}S_{242}$), 726 (${}^{mexh}S_{726}$), 774 (${}^{mexh}S_{774}$), 934 (${}^{mexh}S_{934}$) for pyridoxine HCl

Table 2
Recovery results of thiamine HCl and pyridoxine HCl in their mixtures

Mixtures (μg/ml)		Recoveries (%)																
PYR	THI	Pyridoxine HCl											Thiamine HCl					
		MEXICAN				MEYER							MEXICAN			MEYER				
		$^{mexh}S_{242}$	$^{mexh}S_{726}$	$^{mexh}S_{774}$	$^{mexh}S_{934}$	$^{meyr}S_{160}$	$^{meyr}S_{313}$	$^{meyr}S_{460}$	$^{meyr}S_{601}$	$^{meyr}S_{745}$	$^{meyr}S_{868}$	$^{meyr}S_{997}$	$^{mexh}S_{524}$	$^{mexh}S_{897}$	$^{mexh}S_{1010}$	$^{meyr}S_{482}$	$^{meyr}S_{646}$	$^{meyr}S_{779}$	$^{meyr}S_{896}$
8.0	24.0	97.9	97.0	100.3	96.5	96.3	98.7	106.5	105.3	101.9	102.0	95.0	95.7	104.7	107.5	101.4	102.9	102.8	107.5
16.0	24.0	99.1	96.9	102.2	101.3	106.2	99.8	106.2	100.1	98.7	105.2	99.2	97.8	101.2	102.8	102.5	100.6	100.2	102.8
24.0	24.0	99.9	98.8	99.8	97.5	104.8	99.8	104.8	100.4	97.0	101.0	98.2	98.9	100.1	98.7	103.5	102.2	102.4	98.7
32.0	24.0	101.3	97.8	98.6	101.5	107.4	101.4	107.4	103.9	98.4	104.5	101.6	104.3	98.7	98.8	106.0	103.5	102.5	98.8
40.0	24.0	100.4	99.3	102.6	104.9	104.6	100.1	104.6	101.6	99.7	104.2	100.7	98.9	100.1	100.0	102.6	101.1	100.3	100.0
24.0	8.0	99.9	103.0	103.5	99.2	105.5	100.9	105.5	104.4	101.4	101.0	99.9	100.0	99.0	98.8	97.4	97.1	98.6	98.8
24.0	16.0	99.9	98.1	102.2	104.5	105.7	100.2	105.7	102.4	98.7	106.0	100.7	101.6	99.6	98.6	107.1	102.3	101.4	98.6
24.0	24.0	101.4	96.7	105.5	99.2	104.3	100.5	104.3	101.6	98.3	103.1	100.0	101.3	96.6	99.1	101.5	100.3	100.3	99.1
24.0	32.0	101.4	96.7	104.8	105.2	103.7	100.0	103.7	100.8	98.0	105.9	100.8	100.0	100.1	102.0	103.0	101.9	100.9	102.0
24.0	40.0	99.9	93.3	105.1	99.1	100.4	96.4	100.4	99.4	93.4	98.3	95.8	102.6	96.5	102.2	101.9	101.8	101.2	102.2
Mean		100.1	97.8	102.5	100.9	103.9	99.8	104.9	102.0	98.6	103.1	99.2	100.1	99.7	100.9	102.7	101.4	101.1	100.9
R.S.D.		1.10	2.52	2.29	3.10	3.12	1.39	1.84	1.94	2.39	2.44	2.23	2.47	2.34	2.82	2.57	1.77	1.28	2.82

R.S.D., relative standard deviation; PYR, pyridoxine HCl; THI, thiamine HCl.

Table 3
Commercial vitamin tablet results

Vitamin	Pyridoxine HCl (mg per tablet)										Thiamine HCl (mg per tablet)									
Methods	MEXICAN				MEYER						MEXICAN				MEYER					
Working points	$^{mexh}S_{242}$	$^{mexh}S_{726}$	$^{mexh}S_{774}$	$^{mexh}S_{934}$	$^{meyr}S_{160}$	$^{meyr}S_{313}$	$^{meyr}S_{460}$	$^{meyr}S_{601}$	$^{meyr}S_{745}$	$^{meyr}S_{868}$	$^{meyr}S_{997}$	$^{mexh}S_{524}$	$^{mexh}S_{897}$	$^{mexh}S_{1010}$	$^{meyr}S_{482}$	$^{meyr}S_{646}$	$^{meyr}S_{779}$	$^{meyr}S_{896}$		
Mean	250.1	247.9	247.6	248.6	258.7	254.9	256.7	254.7	248.9	253.3	256.4	247.5	249.0	249.0	249.1	261.8	260.4	260.5		
S.D.	3.41	0.94	2.16	2.91	3.72	3.60	2.28	3.54	3.16	6.72	8.15	2.17	4.98	3.3	3.52	4.25	4.69	8.51		
R.S.D.	1.36	0.38	0.87	1.17	1.44	1.42	0.89	1.39	1.27	2.65	3.18	0.87	2.00	1.3	1.41	1.62	1.80	3.27		
S.E.	1.97	0.54	1.25	1.68	2.15	2.11	1.32	2.04	1.82	3.88	4.71	1.25	2.88	1.9	2.03	2.45	2.71	4.91		
CL ($P = 0.05$)	3.97	1.10	2.52	3.39	4.33	4.20	2.65	4.12	3.67	7.82	9.49	2.52	5.80	3.9	4.09	4.94	5.45	9.90		

S.D., standard deviation; R.S.D., relative standard deviation; S.E., standard error; CL, confidence limit; obtained results are the average of ten experiments for each method.

and at 524 ($^{mexh}S_{524}$), 897 ($^{mexh}S_{897}$) and 1010 ($^{mexh}S_{1010}$) for thiamine HCl.

The amplitude values of the transformed signals were drawn as a graph versus concentrations of thiamine HCl and pyridoxine HCl and a straight line was obtained for both vitamins in the above-indicated points. The regression equations were used to find the concentrations of thiamine HCl and pyridoxine HCl (see Table 1).

The highest values for the regression coefficients (r) were obtained for all regression equations. The detection limit (LOD) (signal to noise 3:1) and the quantitation limit (LOQ) (signal to noise ratio 10:1) were computed by using the data obtained from ten replicate for standard solution of 25 μg/ml of thiamine HCl and pyridoxine HCl. Table 1 contains all parameters of the linear regression equations.

4.4. Validation of the continuous wavelet transform

In order to validate the regression equations different composition mixtures were prepared. We applied the above signal analysing procedure on the synthetic mixture for determination of both compounds and we observed that MEXICAN and MEYER gave us satisfactory results (see Table 2). The validation results indicated us that the calculated calibration at the selected points is useful for determination of the two vitamins in the prepared mixtures.

4.5. Vitamin analysis

Ten tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in 0.1 M HCl in a 100 ml calibrated flask by sonication. The solution was filtered into a 100 ml calibrated flask through Whatman no.42 filter paper and diluted to an appropriate volume with the same solvent. The analysis of the solutions was performed with MEXICAN and MEYER transforms. The experimental results of tablet formulation were summarised in Table 3. The obtained results by using WT are in good agreement with each other as well as with the label values indicated in commercial product.

The excipients in tablets or matrix effect give a constant signal, which is eliminated in the working range of the transformed spectrum. This is a consequence of the WTs as well as it is a matter of choosing the zero-crossing points. In other words our method can be realised in presence of the interference of transformed spectra of both vitamins and in presence of the excipients in tablets.

5. Conclusion

A zero-crossing technique based on CWT was developed to analyse the signal of two vitamins in their mixture. MEXICAN and MEYER followed by a calibration method similarly to zero-crossing method on the transformed signals have been applied to analyse a combination of thiamine HCl and pyridoxine HCl. The transformed signal and the concentration have a very good linear correlation for the measured amplitudes corresponding to the zero crossing points of the working length.

Another advantage of our proposed method is that several calibration graphs can be used in the processes of prediction in the vitamin contents.

The obtained results are reliable in comparison with those given by spectroscopic [33,34], and chemometric [35] methods and they are comparable with those delivered by HPLC method [34].

Taking into account the above results the proposed method is appropriate for the quality control and the routine analysis in the mixtures and commercial products. The software of CWT can be successfully implemented on UV–Vis spectrophotometry as well as to the other analytical instruments.

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