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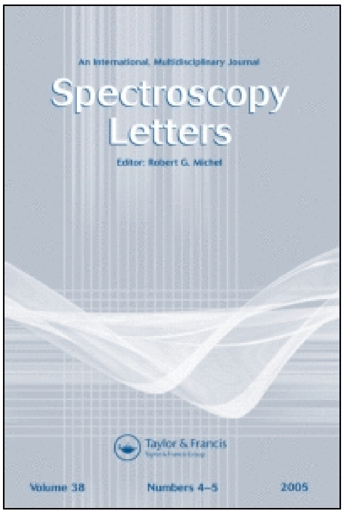

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An Approach to Quantitative Two-Component Analysis of a Mixture Containing Hydrochlorothiazide and Spironolactone in Tablets by One-Dimensional Continuous Daubechies and Biorthogonal Wavelet Analysis of UV-Spectra

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An Approach to Quantitative Two-Component Analysis of a Mixture Containing Hydrochlorothiazide and Spironolactone in Tablets by One-Dimensional Continuous Daubechies and Biorthogonal Wavelet Analysis of UV-Spectra

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ABSTRACT

In this study a new method of quantitative two-component analysis of a mixture of hydrochlorothiazide (**HCT**) and spironolactone (**SP**) in the presence of strongly overlapping signals was achieved by using one-dimensional continuous wavelet analysis. This new method was built on the simultaneous use of both continuous wavelet transform and the zero crossing technique for the quantitative resolution of this binary mixture. A series of solutions, in the concentration range of 2–22 mg/ml for both compounds, in methanol and 0.2 M sodium acetate buffer, pH = 5 (20:80), were considered. The absorption spectra of the standard solutions were recorded in the wavelength range of 215–330 nm. To apply our methods we selected 400 points from the absorption spectra and we subjected the corresponding signal to Daubechies2 (**DAUB2**) and Biorthogonal1.5 (**BIOR1.5**) one-dimensional wavelet transform. In the transformed signals, the amplitude of **HCT** was measured at the position of a zero crossing point of SP and vice versa. Thus simple linear regression analysis can be applied to establish calibrations for both components. These calibrations were validated with synthetic mixtures of HCT and **SP**. *MATLAB 6.5* software was used for one-dimensional wavelet analysis, and the basic concepts about wavelet method are briefly explained. The method developed in this paper is rapid, easy to apply, inexpensive and it is suitable for analysis of the overlapping signals of compounds in their mixtures without any chemical pre-treatment.

Key Words: Daubechies and biorthogonal one-dimensional continuous wavelets; Quantitative resolution; Zero-crossing technique; Hydrochlorothiazide and spironolactone; UV-visible spectrophotometry.

INTRODUCTION

A common problem of spectrometry, often applied in pharmaceutical chemistry, is the simultaneous analysis of two or more components of a mixture, which additionally may contain excipients. To solve this issue, various graphical and numerical methods have been applied, but still the resolution of the multicomponent mixtures has drawbacks in some cases.[1–10]

In these spectral studies, classical derivative and ratio spectra derivative methods were used for the quantitative resolution of the mixtures containing two or more compounds. Because the higher derivative process reduces the peak amplitude, the process of finding zero-crossing points becomes very difficult and the sensitivity of the method is decreased. It may happen that, in some cases, the ratio spectra derivative method leads to infinite values of ratio spectra.

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Wavelet analysis has begun to play a serious role in a broad range of applications, including signal processing, data and image compression, solution of partial differential equations, modelling multiscale phenomena, and statistics. For these reasons, new methods should be discovered and applied to analytical problems. Wavelet transform (**WT**)[11–17] was rapidly developed during the last decade in various branches, e.g. signal processing and de-noising,[18] analysis of electrochemical noise data,[19–21] flow-injection analysis[22] or resolution of simulated overlapped spectra.[23] The basic idea of the continuous wavelet method (**CWT**) is to represent any arbitrary function as a superposition of wavelets. Mallat and Hwang discovered a fast implementation method for the discrete wavelet method (**DWT**)[24] which becomes an effective tool for processing chemical data. It was proven recently that **CWT**, based on the use of the zero-crossing technique, could give better results for the quantitative multicomponent analysis.[25,26]

The purpose of this paper is to apply **DAUB2** and **BIOR1.5** in the simultaneous determination of **HCT** and **SP** in their combination. The obtained results were successfully compared among each other as well as with those given by other literature methods.

Wavelet Transform

Wavelet analysis provides a systematic new way to represent and analyse multiscale structures. Wavelet analysis is also a far-reaching generalisation of orthogonal bases of functions. Their particular new contribution is a systematic way to represent functions on unbounded domains by linear combinations of orthogonal basis functions that are compactly supported and overlapped. **WT** is a procedure that expresses data or operators in terms of different frequency components, and then studies each of them with a resolution matched to its scale.

Continuous Wavelet Transforms

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

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

Here a represents the scale parameter, which is a variable, used to control the scaling, and b denotes the translation parameter controlling the translation. R is the domain of real numbers. A mother wavelet Ψ (λ)

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generates the set of functions $\Psi_{a,b}(\lambda)$ by scaling (or dilatation) and shifting (or translation). **CWT**) of f (λ) is defined as:

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

Here 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 $\Psi_{a,b}(\lambda)$.

The wavelet Ψ is invertible if it satisfies the admissibility condition

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

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

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

where C is given by the following expression

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

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

Discretization of the Continuous Wavelet Transforms

The aim of this method is to reconstruct the function $f(\lambda)$ from samples taken on a discrete grid. In this case we choose the following discretization for a and b

$$a = a_0^j, b = \mathrm{k}b_0a_0^j, \quad \mathrm{j,k} \in \mathrm{Z} \tag{6}$$

where Z represent the set of integers.

The discretized family of wavelets is given by

$$\psi_{j,k}(\lambda) = a_0^{-j/2}\Psi(a_0^{-j}\mathrm{t} - \mathrm{k}) \tag{7}$$

If $a_0 = 2$, $b = 1$, we obtain the dyadic case for which the orthonormal bases exist and reconstruction from transform coefficients is available.

For a given mother wavelet Ψ and appropriate a_o and b_0, we have

$$f(\lambda) = \sum_j\sum_k \mathrm{C}_{j,k}\psi_{j,k}(\lambda) \tag{8}$$

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where

$$C_{j,k} = \langle f(\lambda), \psi_{j,k} \rangle \tag{9}$$

Daubechies constructed orthogonal wavelets with compact support (for more details see. Ref. [14]). Instead of orthogonal wavelet families, one can construct biorthogonal ones.[14] They provide perfect reconstruction of the signal, without redundancy, by using two sets of wavelets: one for the decomposition and another one for the reconstruction.

A mixture of frequencies, shown by its own Fourier transform, composes a wavelet; wavelet coefficients necessarily refer to this mixture of frequencies.

EXPERIMENTAL

Instruments

a) A Shimadzu UV-1600 double beam UV-VIS spectrophotometer connected to a computer having Shimadzu UVPC software and an HP DeskJet 600 printer were used to record the UV-VIS absorption spectra. The analysed signal subjected to **DAUB2** and **BIOR1.5** transformations represents the recorded absorption spectra in the range of 215–330 nm.
b) The calculations and the signal analysis were performed by using *EXCEL* and *MATLAB 6.5*

Commercial Product

A pharmaceutical tablet formulation Aldactazide® tablet (produced by Ali Raif İlaç Ind., Turkey. Batch no. 1C260) containing 25 mg **SP**, 25 mg **HCT** and excipients (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil and magnesium stearate)/per tablet was subjected to **DAUB2** and **BIOR1.5** one-dimensional wavelet analysis.

Reagent

Acetic acid-sodium acetate buffer solution, 0.2 M and pH = 5, was prepared by using analytical-reagent grade reagents. Stock solutions of 100 mg/ml of **HCT** and **SP** were prepared in a solvent containing methanol and 0.2 M acetate buffer solution, pH = 5 (80:20).

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Standard Solutions

A standard series of the solutions containing between 2–20 μg/ml for **HCT** and SP was made from stock solutions. A validation set of 12 synthetic mixtures containing various concentrations of two active compounds was also prepared from the same stock solutions.

Tablet Content Analysis

Ten tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in methanol and 0.2 M acetate buffer solution, pH = 5 (80:20) 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 using **DAUB2** and **BIOR1.5** wavelet transforms in the spectral range of 210.0–289.8 nm.

RESULTS AND DISCUSSION

Experimental Conditions

A standard solution containing **HCT** and **SP** in the concentration range of 2–22 μg/mL was prepared and its spectra were recorded in the range of 215–330 nm. Four hundred points from the original spectra were selected and transferred into MATLAB *6.5* software for the signal analysis process (see Figure 1).

Since the wavelength (λ) plays the role of parameter t in **WT** analysis, then the coefficients C_{ab} can be plotted versus wavelength number (here it runs from 1 to 400). The number of points used in this paper plays a very important role. We observed that if the working range contains many points the peak intensity of the transformed signal and zero-crossing points increased. For a given compound we established an optimization between the number of points and the amplitude of the transformed signal. Our aim was to find many zero-crossing points in such a manner that the transformed amplitude signal was bigger than the analyzed amplitude signal.

We observed that the maximum amplitudes of the transformed signals depend on the applied wavelet family (see Figure 2 and Figure 3). We

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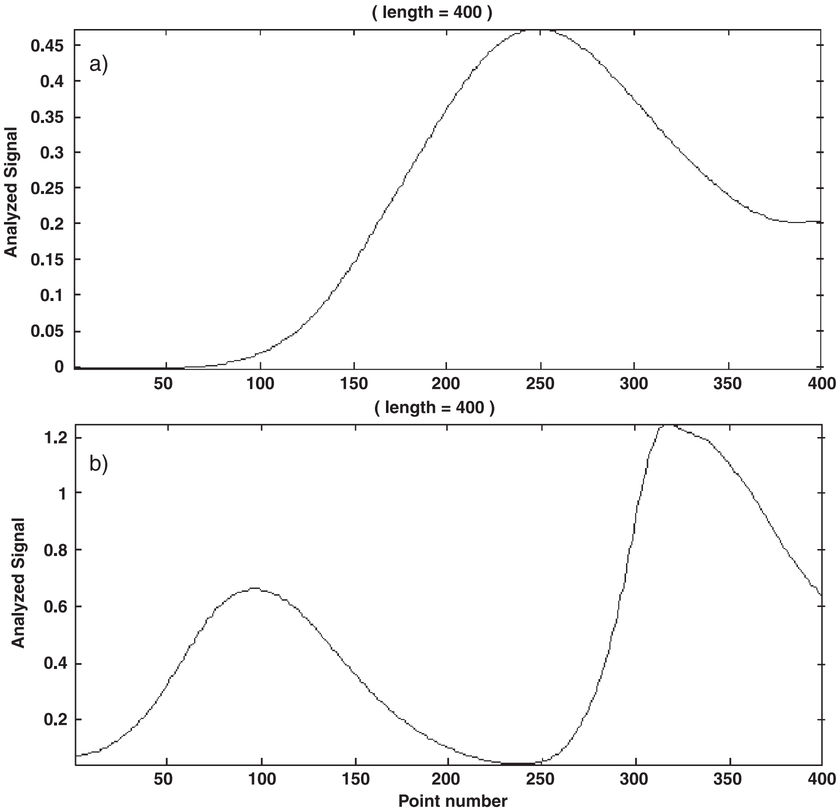

Figure 1. Analyzed spectra of a) 10 µg/ml **SP** and b) 10 µg/ml **HCT** in methanol and 0.2 M acetate buffer solution, pH = 5 (80:20).

investigated our signal with **BIOR1.1** up to **BIOR6.8** and we observed that only **BIOR1.5** fulfils all requirements of our method. Varying the scale parameter from a = 32 and frequency 0.024 to a = 64 and frequency 0.012 we retained the value a = 50 and frequency 0.016. We repeated the same procedure for **DAUB** wavelet, namely we investigated the signal with **DAUB1** up to **DAUB10** and we retained **DAUB2**. Following the same procedure as before we found the optimal values in this case to be $a = 50$ and frequency 0.013.

The second step of our analysis was to apply a zero-crossing technique on the transformed signals.

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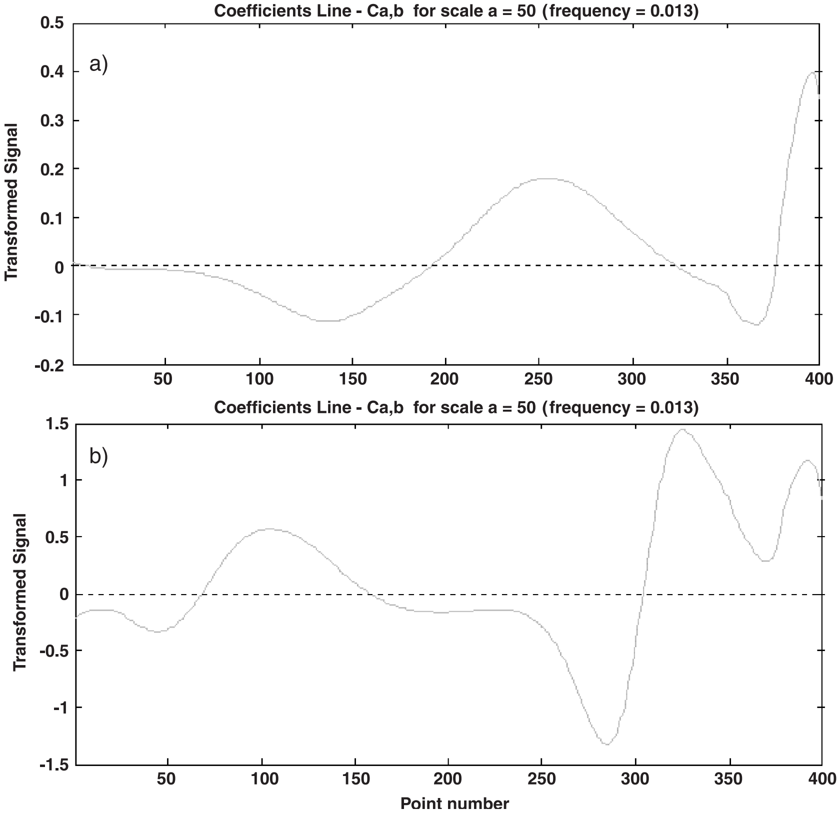

Figure 2. Transformed spectra of a) 10 μg/ml **SP** and b) 10 μg/ml **HCT** by using **DAUB2** transform (a = 50).

CWT Analysis

We have in mind to apply the Beer-Lambert law on transformed signal for a given mixture. If we consider a selected point (i) in the working length, then we obtain:

$$\mathbf{CWT}(\mathbf{S}_{mix,i}) = \mathbf{CWT}(\alpha_i)\mathrm{C}_{\mathbf{HCT}} + \mathbf{CWT}(\beta_i)\mathrm{C}_{\mathbf{SP}} \qquad (10)$$

Here $\mathbf{CWT}(\mathbf{S}_{mix,i})$ is the transformed signal of the binary mixture, $\mathbf{CWT}(\alpha_i)$ and $\mathbf{CWT}(\beta_i)$ are the extinction coefficient of transformed signal for **HCT** and **SP**, respectively and $\mathrm{C}_{\mathbf{HCT}}$, $\mathrm{C}_{\mathbf{SP}}$ are the concentration of **HCT** and **SP**. If

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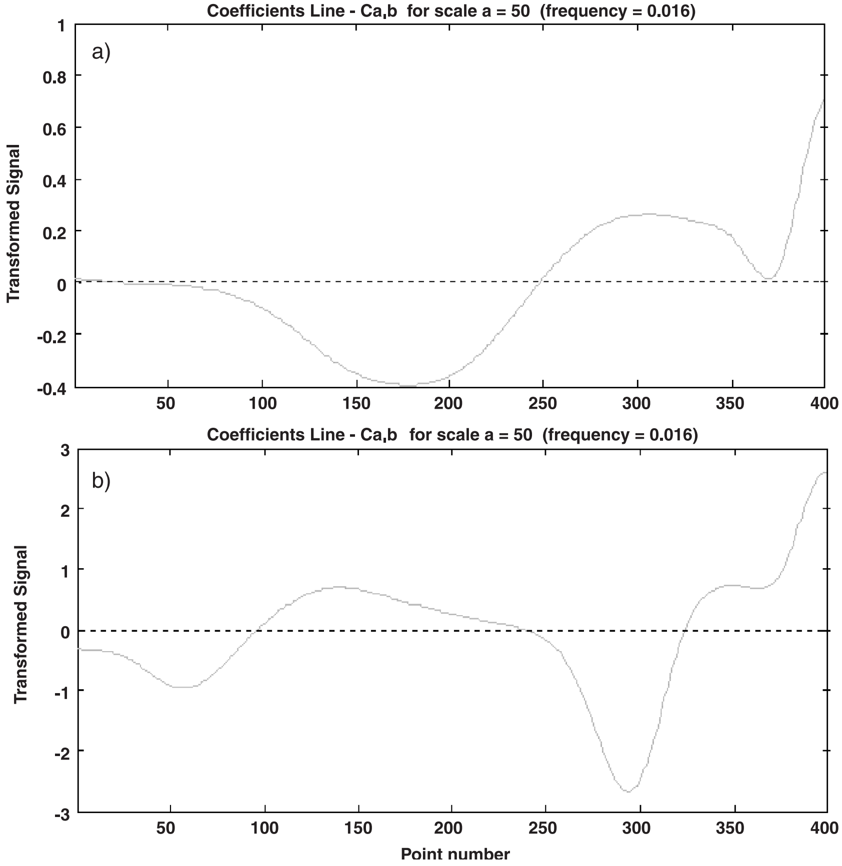

Figure 3. Transformed signal of a) 10 µg/ml **SP** and b) 10 µg/ml **HCT** by using **BIOR1.5** transform (a = 50).

the transformed signal of one of compounds in the binary mixture corresponds to a zero-crossing point in the working length, $\mathbf{CWT}(\alpha_i)\,\mathrm{C}_{\mathbf{HCT}}$ will be zero at selected point (i). This results can be written as:

$$\mathbf{CWT}(\mathbf{S}_{mix,i}) = \mathbf{CWT}(\beta_i)\mathrm{C}_{\mathbf{SP}} \tag{11}$$

From Eq. 11 the amplitude of $\mathbf{CWT}(\mathbf{S}_{mix,i})$ becomes proportional to the concentration of $\mathrm{C}_{\mathbf{SP}}$ in the mixture. A similar procedure can be applied for $\mathrm{C}_{\mathbf{HCT}}$.

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Calibration Equations

The calibration graphs of **HCT** for the wavelet methods were constructed by plotting the transformed signals versus the concentration at the zero crossing points of **SP** and vice versa.

The calibration graphs of **DAUB2** were obtained by measuring the transformed signals at point number 192 ($^{db}S_{192}$) for **HCT** (corresponding to zero crossing point of **SP**) and at point number 158 ($^{db}S_{158}$) for **SP** (corresponding to zero crossing point of **HCT**). By using a similar procedure, the calibration graphs for **BIOR1.5** were constructed by measuring the signal amplitude at point 248 ($^{bior}S_{248}$) for **HCT** and at point 239 ($^{bior}S_{239}$) for **SP** (see Table 1).

The amplitude values of the transformed signals were drawn as a graph versus concentrations of **HCT** and **SP** and a linear regression was performed for both compounds in the above-indicated points. The quantitative analysis of **HCT** and **SP** in the samples was achieved by using the calibration equations (see Table 1). In all calibration equations the linear regression coefficients were higher than 0.9955. Detection limit (**LOD**) (signal to noise ratio 3:1) and quantitation limit (**LOQ**) (signal to noise ratio 10:1) were calculated by using the data obtained from ten replicate measurements for standard solution of 10 μg/ml of HCT and **SP**. All parameters of the linear regression analysis were indicated in Table 1. The linear regression lines obtained at the selected zero-crossing points indicated us that they are suitable for the determination of both compounds.

Validation of Calibrations

In order to validate the above calibration graphs different composition mixtures were prepared. We applied the above signal analysing procedure to the synthetic mixtures for determination of both compounds and we observed that **DAUB2** and **BIOR1.5** gave us satisfactory results (see Table 2). These results show that both methods are effective for the two compounds in synthetic mixtures. During the simultaneous analysis of the binary mixture, no systematic error was observed.

Analysis of Tablet Content

The quantitative determination of **HCT** and **SP** was achieved by using **DAUB2** and **BIOR1.5** methods. The experimental results for the tablet formulation are summarised in Table 3. A good coincidence was reported from the assay results of the commercial preparations in the application the methods of **DAUB2** and **BIOR1.5**.

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Table 1. Calibration graphs data.

Methods	Range µg/ml	Equation	r	S_r	S_m	S_b	LOD µg/ml	LOQ µg/ml
DAUB2	2–22	${}^{db}S_{192} = 0.0000 - 0.0165\ C_{\mathbf{HCT}}$	− 0.9999	0.0873	0.0347	0.0356	0.21	0.71
		${}^{db}S_{158} = 0.0034 - 0.0091\ C_{\mathbf{SP}}$	− 0.9979	0.0651	0.0259	0.0266	0.37	1.25
BIOR1.5	2–22	${}^{bior}S_{248} = -0.0049 - 0.0131\ C_{\mathbf{HCT}}$	− 0.9998	0.0777	0.0309	0.0317	0.54	1.79
		${}^{bior}S_{239} = 0.0004 - 0.0080\ C_{\mathbf{SP}}$	− 0.9956	0.0610	0.0242	0.0249	0.27	0.91

C_{HCT} = HCT concentration (µg/mL).
C_{SP} = SP concentration (µg/mL).
${}^{db}S$ and ${}^{db}S$ = transformed signal of **DAUB2** and **BIOR1.5**.
r = Regression coefficient.
Sr = Standard deviation of regress.
Sm = Standard deviation of slope.
Sb = Standard deviation of intercept.
LOD = Limit of detection.
LOQ = Limit of quantification.

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Table 2. Recoveries of **HCT** and **SP** in their mixtures.

Mixture		Recoveries (%)			
		HCT		**SP**	
HCT	**SP**	**BIOR1.5**	**DAUB2**	**BIOR1.5**	**DAUB2**
Added μg/ml		$^{bior}S_{248}$	$^{db}S_{192}$	$^{bior}S_{239}$	$^{db}S_{158}$
2	10	95.7	104.3	98.2	99.9
6	10	103.4	100.8	101.6	104.4
10	10	103.7	102.8	102.6	100.5
14	10	96.1	100.9	101.8	100.9
18	10	101.8	102.3	101.9	98.9
22	10	99.8	98.2	101.8	100.1
10	2	100.1	100.3	96.6	103.9
10	6	95.4	101.8	102.3	97.4
10	10	97.0	102.5	105.6	97.0
10	14	100.5	103.9	100.7	104.7
10	18	100.5	103.2	101.6	102.4
10	22	95.4	103.3	104.8	100.5
Mean =		99.1	102.0	101.6	100.9
RSD =		0.21	0.70	4.67	0.39

RSD = Relative standard deviation.

Table 3. Commercial tablet results.

Vitamin	**HCT** mg/tablet		**SP** mg/tablet	
Methods	**BIOR1.5**	**DAUB2**	**BIOR1.5**	**DAUB2**
Working points	$^{bior}S_{248}$	$^{db}S_{192}$	$^{bior}S_{239}$	$^{db}S_{158}$
Mean	24.93	25.11	24.82	24.47
SD	0.54	0.52	0.53	0.06
RSD	2.17	2.06	2.13	0.23
SE	0.31	0.30	0.31	0.03
CL(P = 0.05)	0.63	0.60	0.62	0.06

SD = Standard deviation. RSD = Relative standard deviation.
SE = Standard error. CL = Confidence limit.
Obtained results are the average of ten experiments for each method.

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The excipients in the tablets give a constant signal, which is eliminated in the working range of the transformed spectrum. This happens because of definition of the wavelet transforms as well as by the proper choice of the zero-crossing points. In conclusion, our method can be applied in the presence of the interference of transformed spectra of both compounds and in the presence of the excipients in tablets.

CONCLUSION

By combining one-dimensional **CWT** and the zero-crossing technique we propose a new method of quantitative two-component quantitative analysis of mixtures and tablets of **HCT** and **SP**. **DAUB2** and **BIOR1.5** analysis gave successfull results for the commercial tablet formulation. This new approach does not require any pre-chemical separation procedure. 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. Our investigated methods do not require any other complex calibration technique and it is a powerful tool for the resolution of binary mixture systems.

The commercial tablet results of ratio spectra derivative and multi-wavelength regression methods presented in Ref. [27] are 24.91 ± 0.67 (mean (mg) ± SD (standard deviation)) and 24.86 ± 0.72 for **HCT**, and 24.57 ± 0.70 and 24.84 and 0.84 for **SP**, respectively. The commercial tablet results (see Table 3) are reliable in comparison with those delivered in Ref. [27] Since the mean recoveries and its relative standard deviations are 100.5% and ± 0.5% for **HCT**, 100.2% and ± 0.6% for **SP** correspond to the **HPLC** method[28] we conclude that our recovery results are comparable.

Taking into account the advantages of this new method we strongly believe that it is appropriate for use in quality control, and in the routine analysis of mixtures and commercial products involving **HCT** and **SP**. On the other hand the software of **CWT** can be successfully implemented for UV-VIS spectrophotometry as well as for other analytical instruments.

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