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Continuous Wavelet Transforms for Simultaneous Spectral Determination of Trimethoprim and Sulphamethoxazole in Tablets

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A new signal processing approach was developed to improve the simultaneous spectrophotometric determination of trimethoprim (TMP) and sulphamethoxazole (SMX) in their binary mixtures without using any chemical pre-treatment. This approach was essentially based on the continuous wavelet transform (CWT) of the absorption spectra of the target compounds and their samples. A set of continuous wavelet transforms was applied. Biorthogonal with 2.4 order (BIOR2.4), Coiflets with 2 order (COIF2), Daubechies with 3 order (DB3) and Haar (HAAR) were found to be suitable for the quantitative resolution of the two-component mixture containing TMP and SMX compounds. The confirmation of the determination results obtained by applying the BIOR2.4, COIF2, DB3 and HAAR wavelet families was achieved by first derivative spectrophotometry (DS^1) analysis of the same mixtures. The validation of the above-mentioned signal processing methods was tested by analyzing various binary mixtures of the related compounds, and by using the standard addition technique. The simultaneous determination of TMP and SMX in commercial tablets was assessed by using the proposed signal processing tools.

Keywords: Continuous wavelet transforms, Binary mixture, Trimethoprim, Sulphamethoxazole, Tablets

INTRODUCTION

A combination of two antibiotics, TMP and SMX, in pharmaceuticals is used to treat a wide variety of bacterial infections (*e.g.*, middle ear, urine, respiratory and intestinal infections). The combination is also used to prevent and treat a certain type of pneumonia (pneumocystis-type). SMX /TMP ratio in commercial formulations is 5:1. SMX is a sulfonamide antibiotic of broad spectrum. Its antibacterial effect is produced by competing with the natural precursor *p*-aminobenzoic acid in the formation of folic acid [1,2]. Similarly, TMP (2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine) affords a large spectrum of antibacterial action by blocking the production of tetrahydrofolic acid from dihydrofolic acid through binding to and reversibly inhibiting the required enzyme, dihydrofolate reductase [2,3].

Simultaneous determination of TMP and SMX in samples *via* spectrophotometry [4,5], HPLC [6], HPTLC [7], fractional wavelet combined with PLS and PCR [8] and partial least-squares calibration in combination with infrared spectroscopy

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[9] have been reported. The classic spectrophotometric methods have been widely used for the determination of compounds in a given single pharmaceutical formulation. However, these conventional spectrophotometric techniques are likely to yield poor result compared to the simultaneous quantitative analysis of bi- and multi-component mixtures due to the overlapping absorption spectra in the same spectral region.

To overcome this problem, the classical derivative spectrophotometry and its modified versions have been proposed for the mixture analysis. However, in some cases derivative methods cannot give desirable results owing to the interference of main derivative bands with noise peaks as well as diminishing signal/noise ratio for higher order derivatives. Taking into account what was said above; the quantitative resolution of the complex mixture warrants a new signal processing approach.

The wavelet function is orthogonal to all functions which are obtained by shifting the wavelet function to right or left by an integer number. The wavelet function is also orthogonal to all functions which are obtained by dilating the mother by a factor of 2^j and shifting by multiples of 2^j units. It is common knowledge that the wavelet families have the property to efficiently represent functions possessing localized features.

Let us start with a wavelet family denoted by $\Psi(\lambda)$ [10,11]. By scaling and shifting $\Psi(\lambda)$, we get a set of functions denoted by $\Psi_{a,b}(\lambda)$ as indicated below.

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

where *a* denotes the scale parameter, *b* is the translation parameter and R represents the domain of real numbers. For a given signal $f(\lambda) \in L_2(R)$ we define CWT as

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

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

Recently, CWT has been increasingly used as an important signal processing method [10,11] and has been investigated as an alternative to the classical derivative spectrophotometry in the mixture analysis [see for example Refs. [12-22] and the references therein]. CWT affords better quantitative resolution than do the conventional spectral methods [22-25]. For example, the derivative spectrophotometry requires signal smoothing due to the distortion of the derivative signals. In most cases, the derivative band may interfere with the noise band. In this case, we need the de-noising procedure for the obtained derivative signals. Accordingly, the signal/noise ratio in higher order derivative cases can be diminished. In the case of the quantitative spectral resolution of the complex mixtures, the CWT does not require any additional procedure such as smoothing or de-noising of the signals.

The main aim of this study is to develop a new CWT signal processing method for the simultaneous quantitative resolution of the two-component mixture containing TMP and SMX antibiotics. The validation of the proposed CWT-tools combined with zero crossing technique was assessed by analyzing the test solutions corresponding to the synthetic mixtures of TMP and SMX in the different concentration levels. In addition, in order to examine the effect of the tablet excipient on the analysis, the standard addition technique was applied and no interference was observed. Finally, four different wavelet families were applied to the simultaneous quantitative analysis of the two antibiotic compounds in tablets.

EXPERIMENTAL

Apparatus and Software

A Shimadzu UV-160 double-beam UV-Vis spectrophotometer connected to a computer equipped with Shimadzu UVPC software was used for the registration of the absorption spectra of the compounds and their samples in the spectral range of 200-320 nm. The treatment of the absorption spectral data vectors was affected by using the wavelet toolbox in *MATLAB* 7.0 software. Other statistical calculations with the linear regression graphs were performed using Microsoft EXCEL.

Standard Solutions

Stock solutions of the pure compounds (TMP and SMX) were separately prepared by dissolving 25 mg of TMP and SMX in 100 ml ethanol in a calibrated flask. Standard

calibration solutions in the linear concentration ranges between 4.0-28.0 μg ml^{-1} for TMP and 2.0-26.0 μg ml^{-1} for SMX were obtained from the above standard solutions. In order to validate signal processing methods, an independent set containing the different mixtures of TMP and SMX in the above-mentioned concentration ranges was prepared by using the stock solutions prepared beforehand.

Pharmaceutical Tablet Formulation

A tablet dosage form (Bactrim forte®Tablet, Roche Pharm. Ind., Istanbul, Turkey) containing 160 mg TMP and 800 mg SMX per tablet was analyzed by applying the proposed CWT signal processing method. TMP and SMX were obtained from Turkish Pharmaceutical Companies.

RESULTS AND DISCUSSION

Figure 1 indicates the overlapping absorption spectra corresponding to the TMP and SMX in ethanol in the linear concentration range of 4.0-28.0 μg ml^{-1} and 2.0-26.0 μg ml^{-1}, respectively. In order to find the linear dynamic range for each compound, possible concentration levels of the related compounds were tested. The above-mentioned concentration ranges were found to be optimal for the investigated methods. *We know that the simultaneous spectrophotometric determination of the analyzed compounds in their mixture by the classical direct absorbance measurement method is not possible due to the overlapping absorption spectra.* The classical analysis of derivative spectrophotometry does not yield desirable results for all mixtures; therefore, developing new methods and techniques for the quantitative resolution of the two-component mixtures is in order.

The mathematical properties of the wavelet families make our suggested technique more suitable to characterize the overlapping spectral bands. In this study, we applied a set of wavelet families, namely Biorthogonal with the 2.4 order (BIOR2.4), Coiflets with the 2 order (COIF2), Daubechies with 3 order (DB3) and Haar (HAAR) to analyze the overlapping absorption spectra of TMP and SMX. We found out that these wavelet families were appropriate to describe the analyzed mixture after we performed an investigation of all wavelet families within *MATLAB* software. We used DS[1] as a comparative method for the same mixture analysis. We

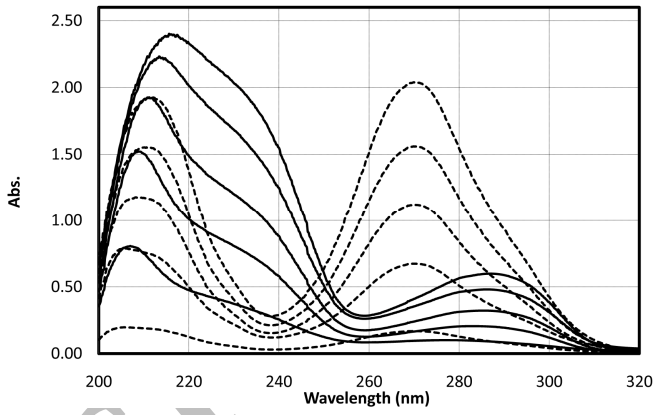

Fig. 1. Absorption spectra of TMP (—); 4.0, 10.0, 16.0, 22.0, 28.0 μg ml^{-1} and SMX (----); 2.0, 8.0, 14.0, 20.0, 26.0 μg ml^{-1}, respectively in ethanol.

know that the derivative amplitudes correlate with the concentrations in the linear calibration structures. In the case of CWT applications, the CWT coefficients were proportional to the concentrations and in the derivative applications. We carried out the linear regression analysis of the relationship between the CWT amplitudes and concentrations.

Signal Processing Methods (Wavelet Families)

In order to select the appropriate wavelet family, all continuous wavelet families in the MATLAB wavelet toolbox were tested whereby BIOR2.4 (a = 96), COIF2 (a = 100), DB3 (a = 76) and HAAR (a = 54) were found to be the optimal signal processing methods to reach the highest recovery results, where a represents the scale factor.

The problem with this approach was that for each wavelet family we had to repeat the calculations within the same family, as well as among the wavelet families, in order to obtain the highest recovery results. In other words, the optimization procedure had to be used which we did manually. It should be noted that the results yielded by the wavelet families also depended on the analyzed data.

After transferring the original absorption data vectors onto the continuous wavelet domain, we processed the data *via* the above-proposed families and under the above-mentioned optimal conditioons.CWT coefficients spectra of TMP and SMX compounds were obtained by plotting the CWT-

coefficients (BIOR2.4-CWT of [Abs.]) *vs*. wavelengths between 205.0 nm and 307.2 nm as shown in Figs. 2-5. Specifically, Fig. 2 depicts the BIOR2.4-CWT spectra of TMP and SMX. This "BIOR2.4-CWT of [Abs.]" denotes the CWT-coefficients obtained by transformation of the absorbance data vectors [Abs.]. By inspection, we observed that the CWT amplitudes at 257.6 nm and 268.0 nm become proportional to the concentration of TMP and SMX, respectively. This crucial result can be specified by considering the form of the utilized wavelet family which also depends on the signal used herein. Two calibration graphs were obtained for the above-mentioned compounds and selected points.

The results obtained by applying the regression analysis to the mathematical relationships between the concentration and the CWT amplitudes measured at the above mentioned wavelengths are displayed in Table 1. We repeated the same

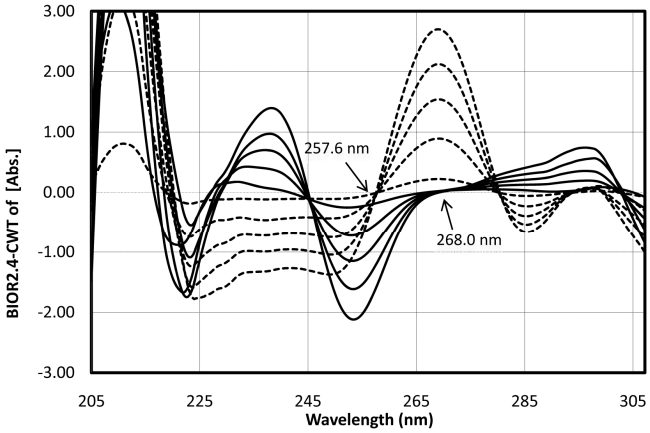

Fig. 2. BIOR2.4-CWT signals of TMP (—); 4.0, 10.0, 16.0, 22.0, 28.0 μg ml^{-1} and SMX (----); 2.0, 8.0, 14.0, 20.0, 26.0 μg ml^{-1}, respectively.

Table 1. Statistical Results Obtained by the Regression Analysis Based on the Relationship between the Concentration and the Measured Amplitudes

	BIOR2.4-CWT		COIF2-CWT		DB3-CWT		HAAR-CWT		DS[1]	
Parameter	TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX
λ (nm)	257.6	268.0	257.0	265.6	253.2	262.2	238.0	259.0	234.6	255.4
Range (μg ml^{-1})	4.0-28.0	2.0-26.0	4.0-28.0	2.0-26.0	4.0-28.0	2.0-26.0	4.0-28.0	2.0-26.0	4.0-28.0	2.0-26.0
m	-5.97×10^{-2}	1.00×10^{-1}	-6.08×10^{-2}	8.84×10^{-2}	-5.17×10^{-2}	4.53×10^{-2}	4.28×10^{-2}	-6.96×10^{-2}	8.53×10^{-3}	-1.44×10^{-2}
n	8.00×10^{-2}	9.76×10^{-2}	1.04×10^{-1}	7.55×10^{-2}	8.68×10^{-2}	1.36×10^{-2}	1.22×10^{-1}	-4.08×10^{-2}	2.58×10^{-2}	-8.13×10^{-3}
r	0.9999	1.0000	0.9999	1.0000	0.9999	0.9999	0.9999	1.0000	0.9999	1.0000
SE(m)	4.14×10^{-4}	5.60×10^{-4}	4.99×10^{-4}	5.05×10^{-4}	4.93×10^{-4}	2.64×10^{-4}	3.85×10^{-4}	3.97×10^{-4}	7.93×10^{-5}	7.04×10^{-5}
SE(n)	7.50×10^{-3}	9.16×10^{-3}	9.04×10^{-3}	8.27×10^{-3}	8.93×10^{-3}	4.32×10^{-3}	6.98×10^{-3}	6.49×10^{-3}	1.44×10^{-3}	1.15×10^{-3}
SE(r)	7.86×10^{-3}	1.06×10^{-2}	9.47×10^{-3}	9.58×10^{-3}	9.35×10^{-3}	5.01×10^{-3}	7.31×10^{-3}	7.52×10^{-3}	1.50×10^{-3}	1.34×10^{-3}
LOD (μg ml^{-1})	0.84	0.61	1.00	0.63	1.16	0.64	1.09	0.63	1.23	0.64
LOQ (μg ml^{-1})	2.81	2.04	3.33	2.09	3.86	2.13	3.65	2.09	4.08	2.15

m = Slope of regression equation, SE(n) = Intercept of regression equation, r = Correlation coefficient, SE(r) = Standard error of correlation coefficient, LOD = Limit of detection, LOQ = Limit of quantitation, SE(m) = Standard error of slope, SE(n) = Standard error of intercept.

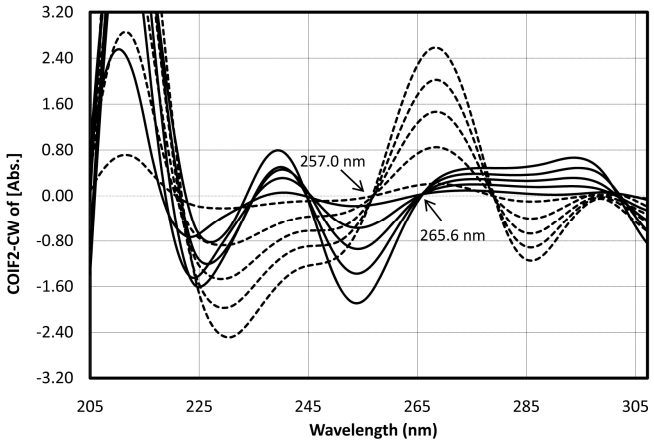

Fig. 3. COIF2-CWT signals of TMP (—); 4.0, 10.0, 16.0, 22.0, 28.0 μg ml^{-1} and SMX (----); 2.0, 8.0, 14.0, 20.0, 26.0 μg ml^{-1}, respectively

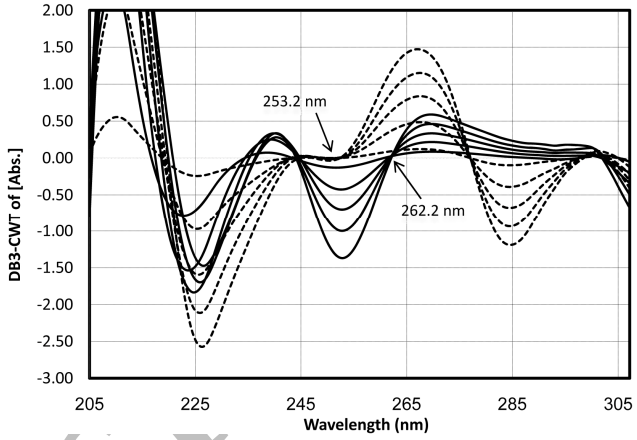

Fig. 4. DB3-CWT signals of TMP (—); 4.0, 10.0, 16.0, 22.0, 28.0 μg ml^{-1} and SMX (----); 2.0, 8.0, 14.0, 20.0, 26.0 μg ml^{-1}, respectively.

procedure for the other three wavelet families the results of which are briefly provided below. In the case of the COIF2-CWT, the desired amplitudes are 257.0 nm and 265.6 nm, as shown in Fig. 3, and the corresponding calibration graphs are illustrated in Table 1.

For the DB3-CWT, we obtained the optimal amplitudes at 253.2 nm and 262.2 nm as it can be seen in Fig. 4, and the appropriate results of the calibration graphs are depicted in Table 1. Finally, the calibration graphs resulting from HAAR-CWT approach for TMP and SMX, indicate the optimal wavelength points at 238.0 nm and 259.0 nm, respectively (see Fig. 5). The numerical results of these calibration graphs can be seen in Table 1. All calibration graphs were used for the quantitative evaluation of the related compounds in their samples.

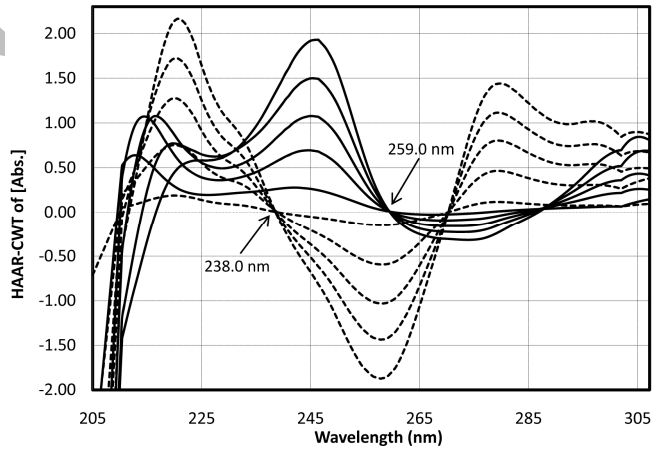

Fig. 5. HAAR-CWT signals of TMP (—); 4.0, 10.0, 16.0, 22.0, 28.0 μg ml^{-1} and SMX (----); 2.0, 8.0, 14.0, 20.0, 26.0 μg ml^{-1}, respectively.

Derivative Spectrophotometry

As it can be seen in Fig. 1, the absorption spectra of TMP and SMX in the linear concentration range of 4.0-28.0 μg ml^{-1} and 2.0-26.0 μg ml^{-1}, respectively, were plotted in the wavelength range of 200-320 nm as shown in Fig. 1. Therefore, the DS[1] of the absorption spectra of the two active compounds and their samples were calculated by using the interval of $\Delta\lambda$ = 5 nm. The obtained first derivative spectra were smoothed by using the interval of $\Delta\lambda$ = 3 nm as indicated in Fig. 6. The next step was to apply the linear regression analysis for TMP and SMX compounds by measuring the derivative amplitude values (d[Abs.]/dλ) at 234.6 nm and 255.4 nm, respectively (see Fig. 6). The output of these calculations was illustrated in Table 1 where satisfactory results are reported. As was mentioned before, we used the obtained regression equations for the simultaneous quantitative evaluation of TMP and SMX drugs in their samples.

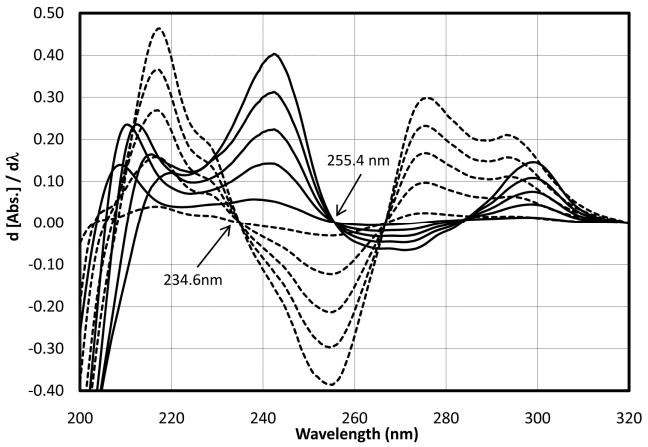

Fig. 6. First derivative of TMP (—); 4.0, 10.0, 16.0, 22.0, 28.0 μg ml^{-1} and SMX (----); 2.0, 8.0, 14.0, 20.0, 26.0 μg ml^{-1}, respectively.

Analytical Validation of Signal Processing Methods

In Table 1, the numerical results of the correlation coefficients (r) reflects a good linearity in the concentration range between 4.0-28.0 μg ml^{-1} for TMP and 2.0-26.0 μg ml^{-1} for SMX. Satisfactory precision values (standard deviation (SD) and the relative standard deviation (RSD)) were observed by applying the proposed four CWT approaches and DS^1 to the analysis of 10 mixture solutions by the dilution of the stock solutions. The percentage recoveries and their corresponding RSD values are illustrated in Table 2. We observed that all investigated wavelet families exhibited better mean recovery values with slight relative standard deviations than did DS^1.

By using the standard deviation of the intercept and slope values of calibration equation, the limit of detection (LOD) and the limit of quantitation (LOQ) were calculated as indicated in Table 1. While applying the standard addition technique, the known amount of TMP and SMX was added to the tablet solution. Therefore, three different concentration levels were used, 5.0, 10.0 and 15.0 μg ml^{-1} for TMP and 2.5, 7.5 and 15.5 μg ml^{-1} for SMX, respectively. This treatment was repeated six times for each concentration level.

The recovery studies were carried out by using the addition technique in order to check the effect of the excipients on the analysis of TMP and SMX in tablets. The standard addition procedure was applied at three different concentration levels (high:15 μg ml^{-1}, medium:10 μg ml^{-1}, low:5 μg ml^{-1} for TMP and high:15.5 μg ml^{-1}, medium:7.5 μg ml^{-1}, low:2.5 μg ml^{-1} for SMT, respectively). Accordingly, the recovery results, standard deviations, and relative standard deviations for the proposed method were calculated which are presented in Table 3. As it can be seen from Table 3, sufficient accuracy and precision are ensured. These numerical results indicate that the proposed signal processing methods were found to be selective for the quantitative analysis of TMP and SMX in tablets because there was no interference from the excipients of tablets.

Analysis of Tablets

Four different CWT and first derivative spectrophotometric methods were successfully applied to the simultaneous quantitative evaluation of TMP and SMX in tablets. The results including mean values, standard deviations, relative standard deviations, standard errors and the confidence level at 95% are illustrated in Table 4. No interference from the tablet excipients in the analysis was observed. Moreover, all the proposed methods yielded successful results for the quality control of the tablets containing TMP and SMX. In order to compare the results obtained by applying CWT and DS^1, the t-test and the F-test were run on the tablet determination results which are indicated in Table 4. The findings reveal that there was no significant difference between the results.

CONCLUSIONS

In this paper, we have introduced a new analytical method based on CWT used for the simultaneous quantitative analysis of TMP and SMX in tablets. We reviewed the literature and found out that there was not any combined use of CWT with zero-crossing technique, namely BIOR2.4-CWT,COIF2-CWT, DB3-CWT,HAAR-CWT and DS^1.The experiments were carried out without any chemical pre-treatment or any preliminary separation step. Wavelet transforms for the signal analysis opened a new avenue for the resolution of the overlapping spectra for the quantitative evaluation of complex mixtures.

We found out that all the investigated wavelet families exhibited better mean recovery values with slight relative

Table 2. Recovery Data Obtained by Applying the Proposed Signal Processing Methods to the Synthetic Binary Mixtures

		Found (μg ml^{-1})									
Binary mix. (μg ml^{-1})		BIOR2.4-CWT		COIF2-CWT		DB3-CWT		HAAR-CWT		DS[1]	
		TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX
TMP	SMX	257.6 nm	268.0 nm	257.0 nm	265.6 nm	253.2 nm	262.2 nm	238.0 nm	259.0 nm	234.6	255.4
4	25	4.07	24.97	4.01	25.13	3.94	25.30	3.96	25.04	3.97	24.31
10	25	9.89	25.18	9.90	25.05	9.91	25.28	9.80	24.99	10.11	24.68
16	25	16.00	25.20	15.79	25.12	16.05	25.01	15.96	24.28	15.72	23.69
22	25	22.43	25.18	22.16	25.30	22.22	25.22	21.64	24.83	21.83	24.36
28	25	28.00	25.01	28.04	24.98	28.08	25.25	27.75	24.80	27.17	24.46
5	2	5.03	1.95	5.19	1.95	5.16	2.03	4.89	1.98	4.75	1.90
5	8	5.03	8.08	5.02	8.19	5.11	8.14	5.00	7.81	4.79	7.88
5	14	5.04	14.04	5.05	14.16	5.07	14.28	4.94	13.91	4.89	14.18
5	20	5.02	20.28	4.88	20.51	5.11	20.57	5.03	20.21	4.87	20.47
5	26	5.00	26.12	4.94	26.79	5.08	26.27	4.90	26.28	4.81	26.45
		Recoveries (%)									
		BIOR2.4-CWT		COIF2-CWT		DB3-CWT		HAAR-CWT		DS[1]	
		TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX	TMP	SMX
		257.6 nm	268.0 nm	257.0 nm	265.6 nm	253.2 nm	262.2 nm	238.0 nm	259.0 nm	234.6 nm	255.4 nm
		101.8	99.9	100.2	100.5	98.5	101.2	99.0	100.2	99.4	97.3
		98.9	100.7	99.0	100.2	99.1	101.1	98.0	100.0	101.1	98.7
		100.0	100.8	98.7	100.5	100.3	100.0	99.8	97.1	98.3	94.8
		102.0	100.7	100.7	101.2	101.0	100.9	98.4	99.3	99.2	97.4
		100.0	100.0	100.2	99.9	100.3	101.0	99.1	99.2	97.0	97.8
		100.7	97.5	103.8	97.3	103.3	101.3	97.7	99.2	95.0	95.1
		100.6	101.0	100.5	102.3	102.2	101.7	100.0	97.6	95.9	98.5
		100.7	100.3	101.0	101.1	101.5	102.0	98.8	99.4	97.8	101.3
		100.5	101.4	97.6	102.5	102.2	102.8	100.5	101.1	97.4	102.4
		100.1	100.4	98.7	103.0	101.6	101.0	97.9	101.1	96.2	101.7
Mean		100.5	100.3	100.0	100.9	101.0	101.3	98.9	99.4	97.7	98.5
RSD		0.88	1.07	1.69	1.62	1.45	0.74	0.95	1.30	1.87	2.66

RSD = Relative standard deviation, Recovery = (calculated concentration/actual concentration) × 100. The first part of the table represents the recovery data and the second part denotes the recoveries.

Table 3. Standard Addition Technique and Recovery Results Obtained by the Proposed Signal Processing Methods

Method	Compound	λ (nm)	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	SD	RSD (%)
BIOR2.4-CWT	TMP	257.6	5.0	4.86	97.1	1.56	1.60
			10.0	9.78	97.8	2.30	2.35
			15.0	14.19	94.6	0.97	1.02
	SMX	268.0	2.5	2.56	102.3	2.88	2.81
			7.5	7.57	100.9	1.42	1.40
			15.5	15.23	98.3	1.86	1.89
COIF2-CWT	TMP	257.0	5.0	5.01	100.2	2.06	2.06
			10.0	10.40	104.0	1.27	1.22
			15.0	14.81	98.8	2.12	2.15
	SMX	265.6	2.5	2.52	100.6	1.94	1.92
			7.5	7.69	102.6	1.20	1.17
			15.5	15.16	97.8	0.81	0.83
DB3-CWT	TMP	253.2	5.0	5.09	101.7	0.83	0.81
			10.0	10.18	101.8	1.43	1.41
			15.0	15.55	103.7	1.04	1.00
	SMX	262.2	2.5	2.55	102.0	1.46	1.43
			7.5	7.7	102.7	0.98	0.95
			15.5	15.8	102.0	1.98	1.94
HAAR-CWT	TMP	238.0	5.0	4.93	98.6	1.60	1.29
			10.0	9.97	99.7	1.29	1.29
			15.0	14.36	95.7	0.97	1.02
	SMX	259.0	2.5	2.56	102.3	1.97	1.92
			7.5	7.4	99.2	1.36	1.37
			15.5	15.2	98.4	1.99	2.02
DS[1]	TMP	234.6	5.0	5.03	100.7	2.04	2.03
			10.0	10.22	102.2	1.73	1.69
			15.0	15.03	100.2	0.99	0.99
	SMX	255.4	2.5	2.47	98.6	2.00	2.03
			7.5	7.4	98.8	2.28	2.31
			15.5	15.1	97.7	1.20	1.23

Six replicates were repeated for each concentration level.

Table 4. Tablet Analysis Results Obtained by the Proposed Signal Processing Methods

Method	drug	λ (nm)	(mg/tablet)	SD	RSD	SE	CL	t-calculated	F-calculated
BIOR2.4-CWT	TMP	257.6	162.0	0.280	0.17	0.09	0.20	2.00	1.70
	SMX	268.0	802.7	0.720	0.09	0.23	0.51	1.46	0.26
COIF2-CWT	TMP	257.0	159.7	0.260	0.16	0.08	0.19	1.58	1.97
	SMX	265.6	805.5	0.230	0.03	0.07	0.16	0.30	2.52
DB3-CWT	TMP	253.2	160.5	0.220	0.14	0.07	0.16	0.33	2.75
	SMX	262.2	805.4	0.210	0.03	0.07	0.15	0.23	3.02
HAAR-CWT	TMP	238.0	160.1	0.250	0.16	0.08	0.18	0.96	2.13
	SMX	259.0	803.5	0.300	0.04	0.09	0.21	1.18	1.48
DS^1	TMP	234.6	160.7	0.365	0.23	0.12	0.26	t-critical = 2.26	F-critical = 3.18
	SMX	255.4	805.1	0.910	0.11	0.28	0.63	(0.05, 9,9)	(0.05, 9,9)

The amounts of active compounds in the commercial tablet formulation are: 160 mg TMP and 800 mg SMX per tablet. n = 10 replicate for each compound determination, SE = Standard error, CL = Confidence limit (p = 0.05).

standard deviations than did DS^1 in the synthetic mixtures. All wavelet families in this work were directly applied to the original absorption spectra while the DS^1 was followed by a smoothing procedure. We conclude that CWT signal processing methods yield better results than do DS^1.On the other hand, results from the four families are comparable with each other. The smoothing process induces additional errors and no systematic procedure exists to treat a given set of data. That is to say, for each set of data a different smoothing procedure is needed. As a result, DS^1 becomes a time-consuming process leading to positive and negative deviations. Our proposed method produces determination comparable to those of HPLC [6]. Finally, all the developed signal processing methods (CWT and DS^1), can be used for the quality control and routine analysis of tablets containing TMP and SMX compounds.

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