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Continuous Wavelet Transform Applied to the Overlapping Absorption Signals and Their Ratio Signals for the Quantitative Resolution of Mixture of Oxfendazole and Oxyclozanide in Bolus

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ABSTRACT

Continuous wavelet transform (CWT) was applied to the overlapping absorption signals and their ratio signals for the quantitative resolution of mixture of oxfendazole (OXF) and oxyclozanide (OXY) in bolus without using any pre-chemical treatment. Mexican hat function (MEXH) and Morlet (MORL) continuous wavelet transforms (MEXH-CWT and MORL-CWT) for signal treatments were found to be suitable among the wavelet families. In the first step MEXH-CWT together with zero crossing technique is directly applied to the overlapping absorption spectra of OXF and OXY drugs and their calibration graphs were obtained by measuring the MEXH-CWT amplitudes at 328.7 nm for OXY and at 309.3 nm for OXF in the wavelet domain of 255-365 nm. In the second step MORL-CWT is used for finding the significant peak resolution of the ratio spectra of OXY and OXF respectively. The maxima and minima of CWT amplitudes and peak-to-peak and zero-crossing techniques were used for the calibration graphs. The method performances of CWT-zero crossing and ratio spectra-CWT techniques were validated by analyzing the synthetic mixtures and by applying the standard addition technique, and successfully accuracy, precision and reproducibility results were reported.

Key words: continuous wavelet transform, zero-crossing technique, ratio spectra treatment, oxfendazol, oxyclozanide

INTRODUCTION

In the field of chemistry the analytical signals for de-noising, compressing, image analysis and other chemical purposes were processed by the wavelets transform or classical Fourier transform(1-3). The chemical instruments produce noises and fluctuations coming from instrumentation recording of a spectrum. Therefore, the cardinal signal of the analyte at lower concentration in samples can interfere with noise peaks. From this reasons, the above mentioned signal analysis methods should be used for the elimination of the noise effect or for increasing the signal to noise ratio. Wavelets are mathematical functions that cut up data into different frequency components, and then study each component with a resolution matched to its scale. They have advantages over traditional Fourier methods in analyzing physical situations where the signal contains discontinuities and sharp spikes. Wavelets were developed independently in the several fields(1-3).

In the analytical studies various graphical and numerical methods have been used for the simultaneous quantitative analysis of the mixtures containing two or more constituents without any separation step. These methods contain some analytical disadvantages such us selectivity and sensitivity, namely these methods don't give correct results in some cases. CWT having flexible and versatile properties for the analytical purposes gives a good resolution of the above mentioned inconvenient states.

As it was pointed out recently, the wavelet methods in combination with zero-crossing and ratio spectra treatment give reliable, sensitivity precise and accurate results for the complex mixture analyses(4-9).

OXF–OXY combination has been used in the veterinary practice as anthelmintic drugs. The quantitation of OXF and OXY in the samples have been performed by derivative and ratio-spectra derivative spectrophotometry(10), chemometric calibration techniques(11) and HPLC(12).

The aim of this study is to develop and apply a new approach based on the combined use of CWT with ratio-spectra and zero-crossing techniques for the overlapping

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signal analysis and simultaneous determination of OXF and OXY in mixtures and in commercially veterinary formulation. The new approaches are based on the measurements of signals at the points corresponding to the maxima and minima (for MORL-CWT), on the measurement of peak to peak (for MORL-CWT) and on the measurement of the signals corresponding to the zero-crossing point (for MEXH-CWT). These methods were validated by analyzing the synthetic mixtures of OXF and OXY and by using the standard addition technique. The experimental results analyzed with the above mentioned approaches were statistically compared with each other and good agreements were reported.

METHOD AND THEORETICAL ASPECTS

I. *CWT Method*

Wavelets, as being a powerful tool of signal processing, have been used in many signal processing domains. The most general principle of the wavelet construction is to use dilations and translations. Commonly used wavelets form a complete orthonormal system of functions with a finite support constructed in such a manner. That is why by changing a scale (dilations) they can distinct the local characteristics of a signal at various scales, and by translations they cover the whole region in which it is studied.

CWT method is a powerful signal processing technique for the overlapping peak resolution and for the significant peak identification. This tool is successfully applied to the spectrophotometric multicomponent analysis of relevant compounds in samples.

Given a mother wavelet [3] Ψ (λ) by scaling (or dilatation) and shifting (or translation) of Ψ (λ) a set of functions $\Psi_{a,b}$ (λ) is obtained as follows

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

where 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. CWT of a given signal f (λ) is defined as:

$$\text{CWT } \{ f(\lambda); a, b \} = \int_{-\infty}^{\infty} f(\lambda)\psi^{*}_{a,b}(\lambda)\, d\lambda = \left\langle f(\lambda), \psi_{a,b} \right\rangle \text{...(2)}$$

Here the superscript * represents the complex conjugate and $\left\langle f(\lambda), \psi_{a,b} \right\rangle$ denotes the inner product of function f (λ) onto the wavelet function $\Psi_{a,b}$ (λ). In addition, the wavelet Ψ is invertible if it satisfies the admissibility condition

$$\int_{-\infty}^{\infty} \frac{|\hat{\Psi}(\omega)|^2}{a} d\omega < \infty \text{ . ... (3)}$$

II. *Morlet and Mexican Hat Function Wavelet Families*

The Morlet wavelet is arguably the original wavelet. Although the discrete Haar wavelets predate Morlet's, it was only as a consequence of Morlet's work that the mathematical foundations of wavelets as a better formulation of time-frequency methods were laid. Conceptually related to windowed-Fourier analysis, the Morlet wavelet is a local periodic wave train. It is obtained by taking a complex sine wave, and by localizing it with a Gaussian envelope (Figure 1).

Analytically, a non-oscillatory function must be subtracted so that the admissibility condition is satisfied.

For a sine of unit frequency inside an envelope of width z_0/π , we obtain

$$\phi(x,z_0) = (\cos 2\pi x + i \sin 2\pi x)\exp\left(-2x^2\pi^2 / z_0^2\right) - \exp\left(-z_0^2/2 - 2x^2\pi^2 / z_0^2\right) \text{ . ... (4)}$$

The selection of z_0 reflects a compromise between localization in time (the Mexican hat isolates a single bump) and in frequency (Fourier's infinite wave train pinpoints the frequency). Clearly, the wavelet transform will have a real and an imaginary part, and it is useful to represent them in polar coordinates: the norm is the magnitude of the transform and, being related to the local energy, is of primary interest, while the polar angle completes the representation. Just as in the case of a Fourier transform, both real and imaginary parts must be known in order to calculate an inverse transform and reconstruct the signal.

The Mexican hat wavelet function is given by the following formula

$$\psi(x) = \left(\frac{2\pi^{-1/4}}{\sqrt{3}}\right)(1 - x^2)e^{-x^2/2} \text{ . ... (5)}$$

This function is proportional to the second deriva-

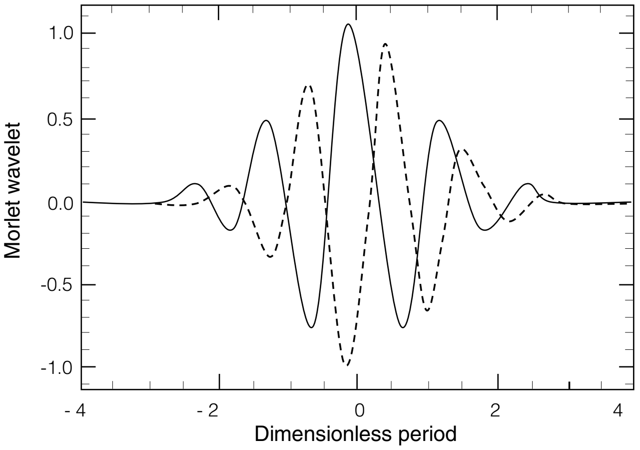

Figure 1. Real (solid line) and imaginary (dashed line) parts of the Morlet wavelet for $z_0 = 5$.

tive function of the Gaussian probability density function (Figure 2).

III. *CWT-zero Crossing Approach*

If a binary mixture of two analytes, X and Y, is considered and if the absorbance value of this binary mixture is measured at λ_i, the following equation can be written as

$$Am_{\lambda i} = \alpha\lambda i\, C_X + \beta\lambda i\, C_Y \ldots\ldots(6)$$

where $Am_{\lambda i}$ is the absorbance of the binary mixture at wavelength λ_i, and the coefficients $\alpha\lambda i$ and $\beta\lambda i$ are absorptivities of X and Y analytes. C_X and C_Y represent the concentrations of analytes.

If CWT is applied to Eq (6), the following equation can be obtained as

$$CWT(Am_{\lambda i}) = CWT(\alpha\lambda i\, C_X) + CWT(\beta\lambda i\, C_Y)\ldots\ldots(7)$$

If $CWT(\alpha\lambda i\, C_X) = 0$, then we obtain the following result

$$CWT(Am_{\lambda i}) = CWT(\beta\lambda i\, C_Y)\ldots\ldots(8)$$

Eq. (8) shows that CWT amplitudes of compound Y in binary mixture are dependent only on the concentration of C_Y and are independent on the concentration of compound X in the binary mixture. As before, the calibration graphs can be obtained by plotting signals versus the concentration of C_Y. This procedure is repeated for X analyte in the binary mixture.

IV. *Ratio Spectra CWT Approach*

If Eq (6) is divided by the spectrum ($A_X^o\lambda i = \alpha\lambda i\, C_X^o$) of a standard solution of one of compounds in binary mixture, the equation (6) becomes:

$$\frac{Am\lambda i}{\alpha\lambda_i C_X^o} = \frac{\alpha\lambda_i C_X}{\alpha\lambda_i C_X^o} + \frac{\beta\lambda_i C_Y}{\alpha\lambda_i C_X^o}\ldots\ldots(9)$$

In this study we applied Mexican hat function as continuous wavelets transform$^{(1\text{-}3)}$ and its application to the ratio spectra is given below.

If CWT is applied to Eq (9), the following equation can be obtained as

$$CWT\left[\frac{Am\lambda i}{\alpha\lambda_i C_X^o}\right] = CWT\left[\frac{\alpha\lambda i}{\alpha\lambda i}\right]\frac{C_X}{C_X^o} + CWT\left[\frac{\beta\lambda i}{\alpha\lambda i}\right]\frac{C_Y}{C_X^o}\ldots(10)$$

If $CWT\left[\frac{\alpha\lambda i}{\alpha\lambda i}\right] = 0$, then we obtain the following result

$$CWT\left[\frac{Am\lambda i}{\alpha\lambda_i C_X^o}\right] = CWT\left[\frac{\beta\lambda i}{\alpha\lambda i}\right]\frac{C_Y}{C_X^o}\ldots\ldots(11)$$

Eq. (11) shows that CWT amplitudes of the ratio spectra of binary mixture are dependent only on the concentration of C_Y and C_X^o and are independent on the concentration of other analyte in the binary mixture. As before, the calibration graphs can be obtained by plotting $CWT\left[\frac{Am\lambda i}{\alpha\lambda_i C_X^o}\right]$ signals versus the concentration of C_Y.

This procedure is repeated for X analyte in the binary mixture.

In this method the concentration C_X in the binary mixture is proportional to the continuous wavelet amplitudes corresponding to minimum and maximum points in the wavelet domain. The calibration graphs for pure X analyte at increasing concentration are obtained by measuring the continuous wavelet amplitudes at the minimum and maximum points in the wavelet domain. In addition, measurement of peak to peak can be used for this application. Making use of the calibration graphs of C_X, the concentration corresponding to X in binary mixture with Y is determined. The concentration of Y compound is determined by a similar procedure.

V. *Signal Analysis Measurement*

The most methods used for the construction of a calibration curve are peak to peak, peak -baseline, peak -tangent and zero-crossing. The measurement method of choice, in practice, would be the one showing the best linear dependence on the concentration of the analyte, a zero or near zero intercept at the origin and be the least influenced by the concentration of other component.

Several signal measurement techniques have been used for the constructions of a calibration graph in the graphical determination methods (Figure 3). The measurement of the amplitudes at the transformed absorption spectra and ratio spectra were carried out by

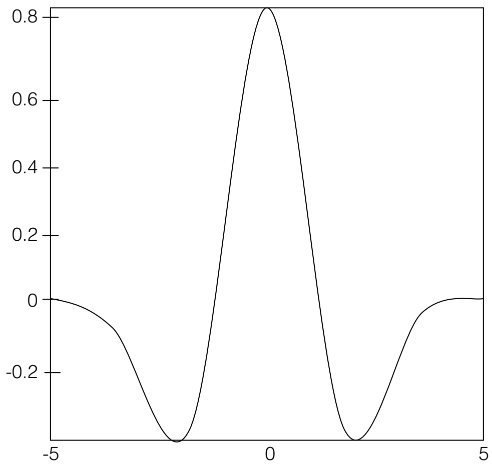

Figure 2. Mexican hat graph corresponding to the equation (5).

using the zero-crossing, peak to peak and maximum and minimum methods as presented in Figure 3[(13-15)].

MATERIALS AND METHODS

I. *Instruments*

A Shimadzu UV-160 double beam UV-Vis spectrophotometer possessing a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer were used to record the absorption spectra. The absorption data were transformed into ASCII files and transferred to *EXCEL* and ratio spectra procedure was performed. After that the transferred data vectors were processed by the proposed CWT approaches. Data treatments, regressions and statistical analysis were performed by using the EXCEL and Wavelet toolbox 3.0 in Matlab 7.0 software.

II. *Commercial Veterinary Formulation*

A commercial veterinary product (OKSAVET® bolus, Vilsan Pharm. Ind., Ankara Turkey, Batch no.10) was assayed. Its declared content was as follows: 300 mg OXF, 600 mg OXY per bolus. OXF and OXY were obtained as a donation from Vilsan Pharm. Ind., Ankara (Turkey).

III. *Standard Solutions*

Stock solution of 50 mg/100 mL OXY and OXF were prepared in 0.1 M NaOH and methanol (50:50, v/v). A standard series in the concentration range of 2-26 μg/mL OXF and 4-28 μg/mL OXY in the same solvent was obtained from the above stock solutions. The synthetic mixture solutions as a validation set in the above working concentration ranges.

IV. *Sample Solutions Preparation*

Twenty boluses were accurately weighed and powdered in a mortar. A sample containing OXF and OXY equivalent to half bolus content was dissolved in 0.1 M NaOH and methanol (50:50, v/v) and made up in 100-mL calibrated flasks. The content of the flask was mechanically shaken for 20 min and filtrated into a 100-mL volumetric flask through a 0.45-μm membrane filter. The resulting solution was diluted to the working concentration range for the application of the methods.

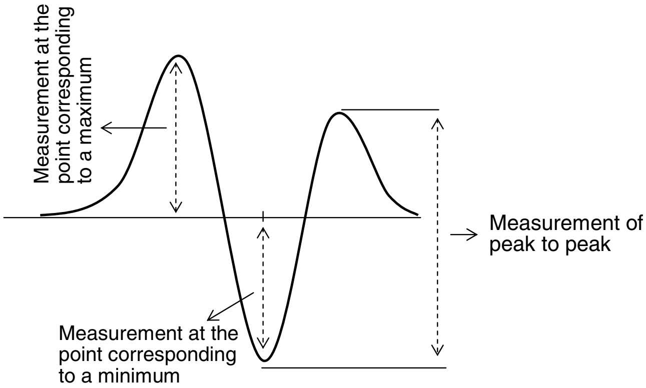

Figure 3. Signal measurement techniques for a calibration graph.

RESULTS AND DISCUSSION

I. *Approach Description*

The analytical signals obtained from UV spectra in the wavelength region of 210-365 nm for standard series solutions of two subjected drugs were prepared for the wavelet analysis (Figure 4). The absorption spectra of sample solutions were also registered in the above mentioned wavelength region to apply the following steps.

The signal treatment of OXY and OXF contains two basic parts. Firstly, MEXH-CWT (a = 150) method was subjected to the original UV absorption spectra (Figure 5). A zero-crossing technique was used to construct the calibration graphs. Secondly, MORL-CWT (a = 125 for OXY and a = 200 for OXF) approach was applied to the ratio-spectra of OXY/OXF and OXF/OXY in samples and calibration graphs were obtained by measuring the analytical amplitude at the points corresponding to maxima and minima of the wavelet signals and by using the measurements of the signals of the peak to peak in the same wavelet signals (Figures 6 and 7).

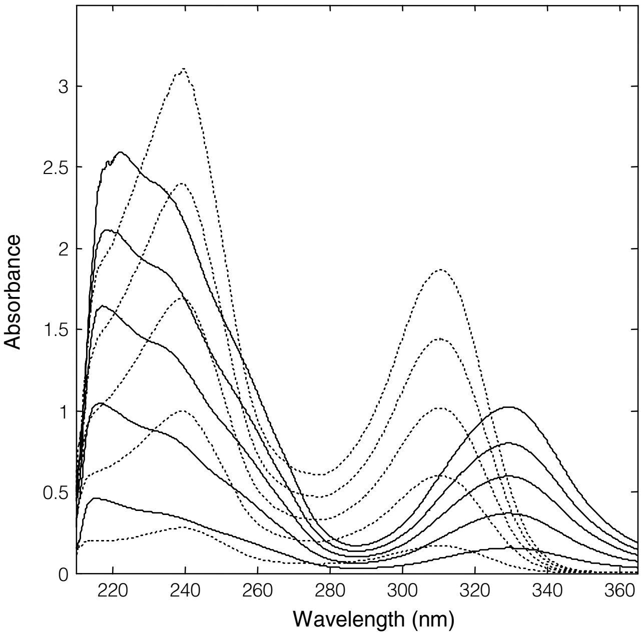

Figure 4. Absorption spectra of 2, 8, 14, 20 and 26 μg/mL OXF (….) and 4, 10, 16, 22 and 28 μg/mL OXY (—) in 0.1 M NaOH and methanol (50:50, v/v).

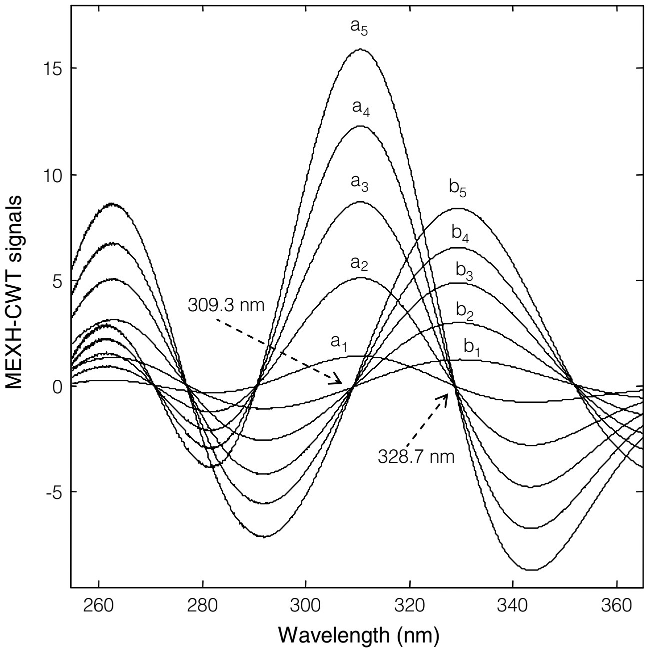

Figure 5. MEXH-CWT spectra of (a_1) 2 µg/mL, (a_2) 8 µg/mL, (a_3) 14 µg/mL, (a_4) 20 µg/mL and (a_5) 26 µg/mL of OXF and (b_1) 4 µg/mL, (b_2) 10 µg/mL, (b_3)16 µg/mL, (b_4) 22 µg/mL and (b_5) 28 µg/mL of OXY by using a = 150 for signal processing.

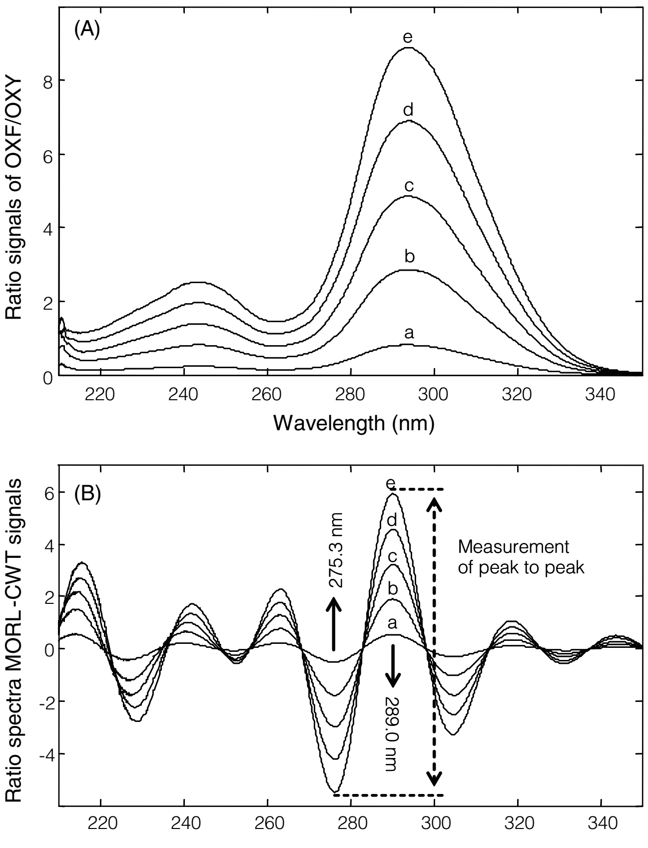

Figure 6. Ratio spectra (A) and their MORL-CWT spectra (B) of (a) 2 µg/mL, (b) 8 µg/mL, (c) 14 µg/mL, (d) 20 µg/mL and 26 µg/mL OXF (when 16 µg/mL OXY was used as a divisor).

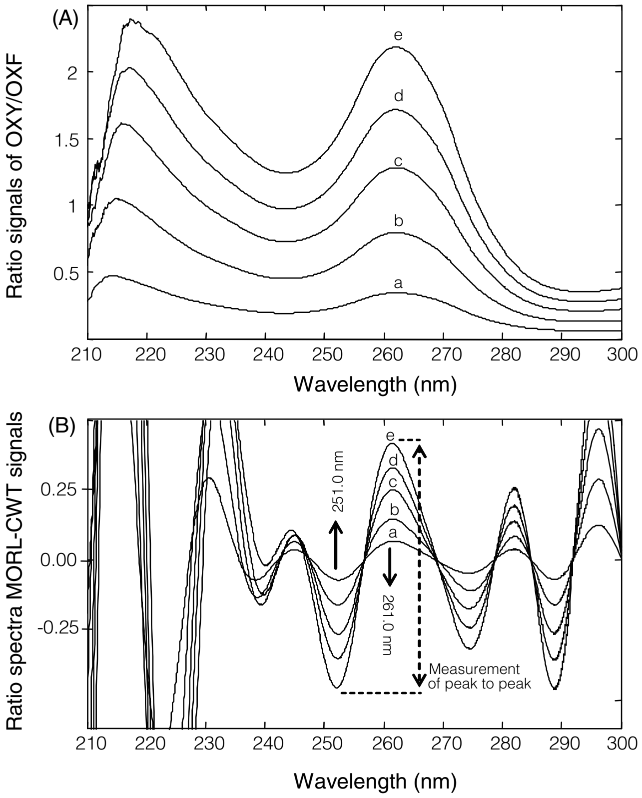

Figure 7. Ratio spectra (A) and their MORL-CWT spectra (B) of (a) 4 µg/mL, (b) 10 µg/mL, (c) 16 µg/mL, (d) 22 µg/mL and 28 µg/mL OXY (when 14 µg/mL OXF was used as a divisor).

II. *MEXH-CWT Processing and Calibration Graph*

The UV-spectra of the standard series and samples solutions in the concentration range of the above mentioned standard solution section were plotted in the range of 210-365 nm and the UV spectra data corresponding to 255-365 nm were selected and transferred to the wavelet domain. The obtained wavelet signals were subjected to all wavelet families available on Wavelet Toolbox in the Matlab 7.0 software and the optimally family was retained. The criteria for obtaining the optimal family are highest sensitivity and selectivity. As a result of this optimization procedure MEXH-CWT (a = 150) was found to be the optimal CWT family. The obtained wavelet coefficients were plotted versus wavelengths and MEXH-CWT spectra were obtained (Figure 5). The next steps were dealing with the calibration graphs. The analytical amplitudes at 309.3 nm for OXF corresponding to the zero-crossing point for OXY and at 328.7 nm for OXY corresponding to the zero-crossing point for OXF were measured to construct the linear regression analysis based on the relation between their peak intensity and concentration.

The obtained calibration graphs and their statistical results from linear regression analysis were presented in Table 1. The results show high correlation coefficients (r) and satisfactory slope, intercept, the limit of detection and the limit of quantitation provided by the application of the MEXH-CWT with the selected optimal zero-crossing points.

This method was applied to the samples in the above mentioned optimized experimental conditions.

III. *MORL-CWT Processing and Calibration Graphs*

The recorded UV-spectra of the standard series and samples solutions in the concentration range of the above mentioned standard solution section for both drugs were plotted in the range of 210-365 nm and the UV spectra data corresponding to 210-350 nm were selected for the ratio-treatment. The obtained UV absorption spectra of OXF standard series and its mixture samples with OXY were divided by the standard UV absorption spectrum of 16 µg/mL OXY and the resulting ratio-spectra were obtained in the spectral range of 210-350 nm (Figure 6A) and these obtained ratio-data vectors were transferred to the wavelet domain.

As in the previous case all wavelet families available on Wavelet Toolbox in the Matlab 7.0 software were applied on the ratio spectra data vectors in the wavelet domain and the optimal family was found to be MORL – CWT (a = 200).

The obtained wavelet coefficients were plotted against wavelengths and the ratio spectra MORL-CWT signals were obtained (Figure 6B).

Under above conditions and taking into account the maximum-minimum techniques the ratio MORL-CWT amplitudes of OXF were measured at 275.3 and 289 nm corresponding to a minimum and a maximum respectively. By using the measurement technique of peak to peak, the analytical amplitudes were measured at 275.3/289.0 for OXF and its mixtures with OXF.

In the application of the above mentioned procedure three calibration graphs for OXF were obtained by using the relation between ratio-MORL-CWT signals and concentration.

The obtained calibration graphs and their statistical results from linear regression analysis were presented in Table 1. We reported high correlation coefficients (r) and satisfactory slope, intercept, the limit of detection and the limit of quantitation for the application of the MORL-CWT with the selected optimal zero-crossing points.

The standard UV absorption spectra of 14 µg/mL OXF was used and the obtained ratio-spectra were plotted in the spectral range of 210-300 nm (Figure 7A) and these obtained ratio-data vectors were transferred into the wavelet domain.

The optimal family was found to be MORL-CWT (a = 125). The obtained wavelet coefficients were plotted in the wavelet domain and the ration spectra MORL-CWT signals are shown in Figure 7B.

By using the maximum-minimum techniques the ratio MORL-CWT amplitudes of OXY were measured at 251.0 and 261.0 nm corresponding to a minimum and a maximum respectively. By using the measurement technique of peak to peak, the analytical amplitudes were measured at 251/261 for OXY and its mixtures with OXF. As a result three calibration graphs for OXF were obtained by using the relation between ratio-MORL-CWT signals and concentration.

The obtained calibration graphs and their statistical results from linear regression analysis are shown in Table 1. We observed that high correlation coefficients (r), satisfactory slope, intercept, the limit of detection and the limit of quantitation were obtained for the application of the MORL-CWT with the selected optimal zero-crossing points. The contents of OXF and OXY in samples were determinate by using the MORL-CWT methods.

IV. *Method Validation*

The linearity of the calibration graphs of OXF and OXY at the selected wavelengths for MEXH-CWT (a = 150) for both drugs and MORL-CWT (a = 200 for OXF and a = 125 for OXY) was verified by high correlation coefficients (r) as shown in Table 1. Ten replicate determinations at the different concentration levels contain-

Table 1. Linear regression analysis and its statistical results

Method		λ nm	Equation	r	SE (a)	SE (b)	SE (r)	LOD (µg/mL)	LOQ (µg/mL)
Zero-crossing	MEXH-CWT	309.3	$S = 0.29716\ C_{OXF} + 0.0447$	0.9999	0.0514	0.0028	0.0539	0.47	1.57
		328.7	$S = 0.5990\ C_{OXY} + 0.2350$	1.0000	0.0272	0.0017	0.0315	0.14	0.46
Ratio spectra	MORL-CWT (a = 200)	275.3	$S = 0.2027\ C_{OXF} + 0.1023$	0.9999	0.0118	0.0007	0.0136	0.61	2.05
		289.0	$S = 0.2163\ C_{OXF} + 0.0967$	0.9999	0.0156	0.0010	0.0181	0.36	1.22
		275.3/289.0	$S = 0.4191\ C_{OXF} + 0.1993$	0.9999	0.0250	0.0015	0.0290	0.18	0.60
	MORL-CWT (a = 200)	251.0	$S = 0.0149\ C_{OXY} + 0.0011$	0.9991	0.0068	0.0004	0.0071	0.56	1.86
		261.0	$S = 0.0146C_{OXF} + 0.0054$	0.9990	0.0072	0.0005	0.0071	0.60	1.99
		251.0/261.0	$S = 0.0296\ C_{OXF} + 0.0044$	0.9994	0.0103	0.0006	0.0108	0.55	1.82

S: analytical signals; SE (a): standard error of intercept; SE (b): standard error of slope; SE (r): standard error of correlation coefficient; LOD: limit of detection; LOQ: limit of quantitation.

ing binary mixtures were used to test the precision of the proposed wavelet methods. The relative standard deviations were found between 3.23% and 1.63%, and these values demonstrate satisfactory reproducibility of the methods as presented in Table 2.

The accuracy of the applied methods was tested by analyzing the same binary mixtures. The mean percentage recovery results were observed between 98.2% and 102.4% (Table 2). A good coincidence of the obtained experimental results was observed for the validation of the methods. No degradation product, interference and systematical errors were observed during the analysis procedure.

In order to observe the matrix effect in the commercial samples, the recoveries of the methods were performed by using the standard addition technique and its calculated results, namely the mean % recovery, standard deviation (SD) and relative standard deviation (RSD) were presented in Table 3. These results were obtained on the average of five replicates for each drug and a good agreement was observed for the standard addition assay results using these methods. As it can be seen in this table no matrix effect was observed during the analysis.

The limit of detection (LOD; signal-to-noise ration 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 14 μg/mL OXF and 16 μg/mL OXF. These results can be seen in Table 1.

V. *Commercial Veterinary Formulation Analysis*

The MEXH-CWT and ratio MORL-CWT methods

Table 2. Recovery data obtained by applying CWT methods to the synthetic mixtures

Binary mixture (μg/mL)		Recovery (%)							
		OXF				OXY			
		Ratio spectra (divisor 16 μg/mL OXY) MORL-CWT (a = 200)			Zero-crossing MEXH-CWT	Ratio spectra (divisor 14 μg/mL OXF) MORL-CWT (a = 125)			Zero-crossing MEXH-CWT
OXF	OXY	275.3 nm	289.0 nm	275.3/289.0	309.3 nm	251.0 nm	261.0 nm	251.0/261.0	328.7 nm
2	20	106.1	105.3	101.7	94.7	96.3	100.0	98.1	99.1
8	20	101.0	104.0	102.6	100.6	101.6	102.5	102.0	101.1
14	20	101.7	102.4	102.1	101.5	95.6	95.2	95.4	99.9
20	20	100.3	101.2	100.8	100.4	98.1	101.5	99.8	99.4
26	20	95.4	100.1	96.4	101.5	97.4	100.0	98.7	101.4
10	4	100.9	100.9	100.9	101.2	102.5	107.8	102.6	95.4
10	10	98.6	99.7	99.2	97.3	96.3	98.9	96.0	100.0
10	16	97.9	99.8	98.9	97.2	96.3	102.3	98.2	99.4
10	22	100.3	103.0	101.7	99.1	98.7	102.5	100.6	99.3
10	28	103.6	107.2	105.5	98.8	103.1	99.2	101.2	99.4
Mean		100.6	102.4	101.0	99.2	98.6	101.0	99.2	99.3
RSD		2.95	2.48	2.42	2.27	2.85	3.23	2.61	2.32

Table 3. Recovery results obtained by standard addition technique

	OXF				OXY			
	MORL-CWT (a = 200)			MEXH-CWT	MORL- CWT (a = 125)			MEXH-CWT
	275.3 nm	289.0 nm	275.3/289	309.3 nm	251 nm	261 nm	251/261	328.7 nm
	97.8	99.7	99.7	102.8	101.7	102.8	102.2	101.4
	95.2	96.9	97.5	99.8	100.8	100.0	100.4	99.7
	97.4	99.0	98.2	101.1	95.4	93.9	94.7	98.2
	93.9	95.3	94.6	98.7	99.6	97.1	98.4	98.5
	95.9	97.4	97.9	101.1	98.5	98.8	98.6	100.1
Mean	96.0	97.7	97.6	100.7	99.2	98.5	98.9	99.6
SD[a]	1.45	1.55	1.67	1.38	2.19	2.96	2.52	1.15
RSD	1.51	1.59	1.72	1.37	2.21	3.00	2.55	1.16

[a] SD: standard deviation.

were successfully applied to the quantitative determination of OXF and OXY in commercial veterinary formulation. The obtained experimental results for the commercial preparation were presented in Table 4.

Statistical values of mean value, SD (standard deviation), RSD (relative standard deviation), SE (statistical value), CL (P = 0.05) (confidential limit) are summarized in Table 4. As it can be seen a good coincidence observed between the experimental results and the claimed label using all the wavelet methods for the veterinary formulation.

VI. *Significant Test of Results*

For a comparison, one-way ANOVA test was applied to the assay results of the commercial veterinary formulation by proposed methods. ANOVA test results were summarized in Table 5. The theoretical F- values indicate that there was no significant difference among the results of the methods. Besides, the numerical values of all statistic tests indicated that the investigated approaches are suitable for the determination of OXY and OXF in the commercial formulation.

CONCLUSIONS

This study reports a combined use of CWT with traditional graphical techniques (ratio-spectra and zero-crossing technique) for the simultaneous determination of OXF and OXY in samples without any separation procedure. In addition, several peak measurement techniques such us maximum and minimum peak amplitude and peak to peak were used to apply the MEXH-CWT and MORL-CWT to the resolution of the subjected binary mixtures.

CWT approach applied to the absorption spectra and the ratio spectra provided higher peak amplitudes, fewer noises, sharp peaks and it does not produce any diminishing of signal intensity in higher derivative calculations as well as the derivative spectroscopy. On the other hand, chemometric calibration technique poses abstract mathematical theory and difficult applicability such us calibration concentration matrix design that takes a period of time. However, the algorithm of CWT is very fast and its application becomes simpler for researchers in the analytical applications. Besides, our proposed hybrid approach based on CWT with zero-crossing and ratio procedure does not need any optimization such us scaling factors, sampling interval and smoothing function as well as derivative spectroscopy. In case of PLS and PCR algorithms we believe that their applications need basic and advanced mathematical background because a mixture analysis also needs difficult preliminary preparation of calibration set and validation set with respect to the content of compounds in samples. In some cases these preparations of calibration set and prediction set get unsolvable problems for mathematical treatments due to the lack of inverse of some involved matrices. Our hybrid approaches do not require any preliminary preparations for calibration and prediction sets.

Therefore, we conclude that our hybrid approaches are more reliable and efficient for the resolution of overlapping spectra than classical spectrophotometric and chemometric calibrations.

The results obtained in this study strongly indi-

Table 4. Experimental results obtained from commercial veterinary formulation by the proposed CWT approaches

	OXF				OXY			
	Ratio spectra (divisor: 16 μg/mL OXY)			Zero-crossing	Ratio spectra (divisor: 14 μg/mL OXF)			Zero-crossing
	MORL-CWT (a = 200)			MEXH-CWT	MORL-CWT (a = 125)			MEXH-CWT
	275.3 nm	289.0 nm	275.3/289	309.3 nm	251 nm	261 nm	251/261	328.7 nm
Mean	297.7	295.5	295.7	299.9	591.5	595.3	598.0	596.9
SD	2.52	4.68	4.14	0.90	3.40	5.12	2.97	5.56
RSD	0.85	1.58	1.40	0.30	0.57	0.86	0.50	0.93
SE	0.95	1.77	1.56	0.34	1.28	1.94	1.12	2.10
CL (P = 0.05)	1.87	3.47	3.06	0.67	2.52	3.79	2.20	4.12

[a]Label claimed: commercial veterinary product, OKSAVET® bolus: 300 mg OXF and 600 mg OXY per bolus.
[b]The obtained results are the average on the seven replicates.

Table 5. One-way ANOVA test for the commercial veterinary formulation results obtained by the proposed CWT approaches

Source of variation	Sum of squares		Degree of freedom		Mean squares		Calculated F-value		F-crit
Drug	OXF	OXY	OXF	OXY	OXF	OXY	OXF	OXY	OXF and OXY
Between groups	89.93	167.79	3		30.0	55.93	2.60	2.88	3.01
Within groups	277.04	465.41	24		11.5	19.39			
Total	366.97	633.20	27						

cate that the above subjected method gives comparable results with the reported HPLC method. Namely, the mean recovery and the relative standard deviation were observed between 100.4-108%, 0.90-1.68% for chemometric methods(11), 98.8- 100.4% and 1.73-1.89% for HPLC(12), 96.0-100.7% and 1.51-3.00% for our approach.

We conclude that the new combined CWT methods can be applied successfully to the routine quality control of the commercial veterinary formulation containing OXF and OXY in bolus.

REFERENCES

1. Walczak, B. 2000. Wavelets in Chemistry. Elsevier Press. Amsterdam, The Netherlands.
2. Daubechies, I. 1992. Ten Lectures on Wavelets. Society for Industrial and Applied Mathematics. Philadelphia.
3. Ma, C. X. and Shao, X. G. 2004. Continuous wavelet transform applied to removing the fluctuating background in near-infrared spectra. J. Chem. Info. Compt. Scienc. 44: 907-911.
4. Dinc, E. and Baleanu, D. 2004. Multicomponent quantitative resolution of binary mixtures by using continuous wavelet transform. J. AOAC Int. 87: 360-365.
5. Dinc, E. and Baleanu, D. 2004. Application of the wavelet method for the simultaneous quantitative determination of benazepril and hydrochlorothiazide in their mixtures. J. AOAC Int. 87: 834-841.
6. Dinç, E., Ozdemir, A. and Baleanu, D. 2005. An application of derivative and continuous wavelet transforms to the overlapping ratio spectra for the quantitative multiresolution of a ternary mixture of paracetamol, acetylsalicylic acid and caffeine in tablets. Talanta 65: 36-47.
7. Dinç, E., Baleanu, D. and Üstündağ, Ö. 2003. 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. Spectr. Lett. 36: 341-355.
8. Dinç, E., Özdemir, A. and Baleanu, D. 2005. Comparative study of the continuous wavelet transform, derivative and partial least squares methods applied to the overlapping spectra for the simultaneous quantitative resolution of ascorbic acid and acetylsalicylic acid in effervescent tablets. J. Pharm. Biomed. Anal. 37: 569-575.
9. Dinç, E. and Baleanu, D. 2005. A new fractional wavelet approach for the simultaneous determination of ampicillin sodium and sulbactam sodium in a binary mixture. Spectrochimica Acta A 63: 631-638.
10. Dinç, E. and Onur, F. 1997. Comparative study of the ratio spectra derivative spectrophotometry, derivative spectrophotometry and Vierordt's method applied to the analysis of oxfendazole and oxyclonazide in a veterinary formulation. Analusis 25: 55-59.
11. Dinç E. and Kanbur, M. 2002. Spectrophotometric multicomponent resolution of a veterinary formulation containing oxfendazole and oxyclozanide by multivariate calibration-prediction techniques. J. Pharm. Biomed. Anal. 28: 779-778.
12. Khan, A. R., Akhtar, M. J., Mahmood, R., Ahmed, S. M., Malook, S. and Iqbal, M. 2000. LC assay method for oxfendazole and oxyclozanide in pharmaceutical preparation. J. Pharm. Biomed. Anal. 22: 111-114.
13. Battenbury, J. M. 1981. Amino Acid Analysis. Halsed-Willey Press. Chichester, U. K.
14. O'Haver, T.C. and Begley, T. 1981. Signal-to-noise ratio in higher order derivative spectrometry. Anal. Chem. 53: 1876-1878.
15. Morelli, B. 1998. Zero-crossing derivative spectrophotometric determination of mixtures of cephapirin sodium and cefuroxime sodium in pure form and in injections. Analyst 113: 1077-1082.