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RESEARCH ARTICLE

Simultaneous Determination of Atenolol and Chlorthalidone in Tablets by Wavelet Transform Methods

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SUMMARY

In this paper two graphical methods based on Coiflet 5 continuous wavelet transform (COIF-CWT) and Mexican Hat function continuous wavelet transform (MEXH-CWT) were developed and applied to the UV absorption spectra of binary mixtures containing atenolol (ATE) and chlorthalidone (CHL) in the presence of strongly overlapping signals. In order to obtain appropriate transformation of UV spectra of analyzed compounds, several values of scaling parameter (a) were tested. The optimal values of the scale parameters were found to be a= 120 for COIF-CWT and a=128 for MEXH-CWT. In the CWT applications, calibration curves for ATE and CHL were found to be linear in the range of 20-120 µg/mL and 15-60 µg/mL, respectively. An algorithm written in Matlab 7.10 was used for the one dimensional wavelet analysis. The validation of the mentioned signal processing methods was performed by analyzing various binary mixtures of ATE and CHL. The proposed signal processing methods were then applied to the analysis of commercial tablets. The methods presented in this paper are rapid, easy, inexpensive and do not need any separation process or preliminary treatments for the analysis of the overlapping UV spectra of ATE and CHL in their samples.

Key Words: *Drug analysis, chemometrics, wavelet transform, spectrophotometry, atenolol, chlorthalidone.*

Tabletlerdeki Atenolol ve Klortalidonun Dalgacık Dönüşümü Yöntemleriyle Eşzamanlı Tayini

ÖZET

Bu çalışmada Coiflet sürekli dalgacık dönüşümü (COIF-SDD) ve Mexican Hat function sürekli dalgacık dönüşümüne (MEXH-SDD) dayalı iki grafiksel metod geliştirilmiş ve atenolol (ATE) ve klortalidon (CHL) içeren ikili karışımların üstüste çakışan UV absorbsiyon spektrumlarına uygulanmıştır. Analiz edilen bileşiklerin UV spektrumlarının uygun dönüşümleri için farklı skala (a) değerleri test edilmiştir. COIF-SDD ve MEXH-SDD için optimal skala değerleri sırasıyla a=120 ve a=128 olarak bulunmuştur. SDD uygulamalarında, ATE ve CHL için kalibrasyon eğrilerinin doğrusal aralıkları sırasıyla 20-120 µg/mL ve 15-60 µg/mL olarak bulunmuştur. Tek boyutlu dalgacık analizleri için Matlab 7.10 kullanılarak yazılan özel bir algoritma kullanılmıştır. Bahsi geçen sinyal işleme yöntemlerinin validasyonları ATE ve CHL içeren ikili karışımların analizi yapılarak gerçekleştirilmiştir. Sunulan sinyal işleme yöntemleri daha sonra ticari tabletlerin analizi için uygulanmıştır. Bu makalede sunulan yöntemler ATE ve CHL moleküllerinin çakışan UV spektrumlarının analizi için herhangi bir ayrım veya ön işlem gerektirmeyen hızlı, kolay ve ucuz yöntemlerdir.

Anahtar Kelimeler: *İlaç analizi, kemometri, dalgacık dönüşümü, spektrofotometri, atenolol, klortalidon.*

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INTRODUCTION

Atenolol (ATE) is a selective beta-blocker which is indicated for the treatment of hypertension. Chlorthalidone (CHL) is a diuretic agent which reduces plasma volume. ATE and CHL work by different mechanisms of action and have additive impacts on the cardiovascular system. Combination of ATE and CHL is used to keep blood pressure under control in hypertensive patients (Seedat, 1980; Pareek et al., 2008).

Quantitative analysis of complex mixtures is mostly problematic and usually requires sophisticated instrumentation or chemical/physical pretreatments. In analytical chemistry and related branches, the development of fast and easy methods for the analysis of these samples is an important task. Several numerical (Wang, Shi, Chen, & Cheng, 2000; E. Dinç & Baleanu, 2002) and graphical methods (Salinas, Nevado, & Mansilla, 1990; Dinç & Onur, 1998) were developed for the spectral resolution of mixtures but these approaches may not give expected analysis results if there is strong spectral interference. For example, in the application of derivative and ratio spectra derivative methods, a good resolution of a binary system may not be achieved. Also, finding the zero-crossing points becomes very difficult or impossible in high-order derivative methods since the peak amplitude decreases with higher order derivative procedures. Wavelet transform (Daubechies, 1992; Meyer & Salinger, 1992) is another approach for the spectral resolution of mixtures containing two or more compounds. The principal of continuous wavelet transformation (CWT) is the representation of an arbitrary function as superposition of wavelets. CWT is a fast and effective approach to process chemical data and gives better results for the spectral resolution of mixtures when used in combination of zero-crossing technique. (Dinç & Baleanu, 2003) Some typical applications of CWT methods to the drug analysis can be found in the literature. (Dinç et al., 2003; Sohrabi et al., 2010; Mohammadpour et al., 2010; Dinç et al., 2013)

This paper presents the development of two different CWT methods for the analysis of a solid dosage form containing two drug substances. COIF-CWT and MEXH-CWT families were applied to the simultaneous determination of ATE and CHL in commercial tablets.

Theoretical background

A wavelet transform decomposes a signal function (a vector) into a simpler, fixed building blocks at different scales and positions. 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 function which are obtained by dilating the mother by a factor of 2^j and shifting by multiples of 2^j units.

Let us start with a wavelet family denoted by $\psi(\lambda)$, (Daubechies, 1992; Walczak, 2000). By scaling and shifting $\psi(\lambda)$, we get a set of functions denoted by $\psi_{a,b}(\lambda)$ as seen below:

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

where a represents the scale parameter, b denotes to the translation parameter. The action of a CWT on the function $f(x)$ is given below;

$$CWT\{f(x); a, b\} = \int_{-\infty}^{\infty} f(x)\psi^{*}_{a,b}(x)dx = f(x), \psi_{a,b}$$

here the superscript * denotes the complex conjugate and $\langle f(x), \psi_{a,b} \rangle$ is the inner product of function $f(x)$ onto the wavelet function $\psi_{a,b}(\lambda)$ (Erdal Dinç & Baleanu, 2007; E. Dinç, Kadioğlu, Demirkaya, & Baleanu, 2011; Dinç, 2013).

MATERIAL AND METHODS

Instrumentation and software

UV absorption spectra of all samples were recorded by Shimadzu UV- 2550 UV-VIS double beam spectrophotometer which was connected to personal computer equipped with Shimadzu UV-Probe (Shimadzu, Japan). The original spectra were recorded between 245-305 nm with an interval of 0.05 nm. The spectral data was transferred Microsoft Excel and were processed by specially-written m-files in Matlab 7.10 (Mathworks, USA).

Standard solutions

The stock solutions of ATE and CHL were individually prepared in methanol with a concentration of 200 μg/mL. The calibration solutions of the compounds were prepared by dilution of the stock solutions in the concentration range of 20-120 μg/mL and 15-60 μg/mL for ATE and CHL, respectively. An independent validation set of 12 binary mixture solutions was also prepared from the stock solutions in the above concentration ranges. The inter-day and intra-day sample solutions were prepared by required dilution of stock solution to obtain three different concentration levels.

Procedure of commercial sample analysis

A film coated tablet dosage form (Tenoretic Tablet, Astra Zenaca, Turkey) containing 100 mg ATE and 25

mg CHL was analyzed by the proposed methods. 20 tablets were accurately weighed and finely powdered in a mortar. An amount powder equal to one tablet mass was dissolved in 100 mL of methanol. After 30 min of mechanically shaking, the solution was filtered by using a membrane filter (Sartorius Minisart with a pore size of 0.45 µm). The UV absorption spectrum of the sample solution was recorded after required dilution. This procedure was repeated ten times.

RESULTS AND DISCUSSION

In order to define the linear concentration range for each compound, possible concentration levels of ATE and CHL were investigated. The concentration ranges of 20–120 µg /mL and 15-60 µg/mL were found to be appropriate for ATE and CHL, respectively. The absorption spectra corresponding to ATE and CHL in methanol in the above-mentioned linear ranges were illustrated in Figure 1. Due to their overlapping absorption spectra, the spectrophotometric determination of the related drugs in their binary mixture was not possible by conventional absorbance measurement. Therefore, in this study, signal processing approach was used for the quantitative resolution of binary mixtures containing ATE and CHL.

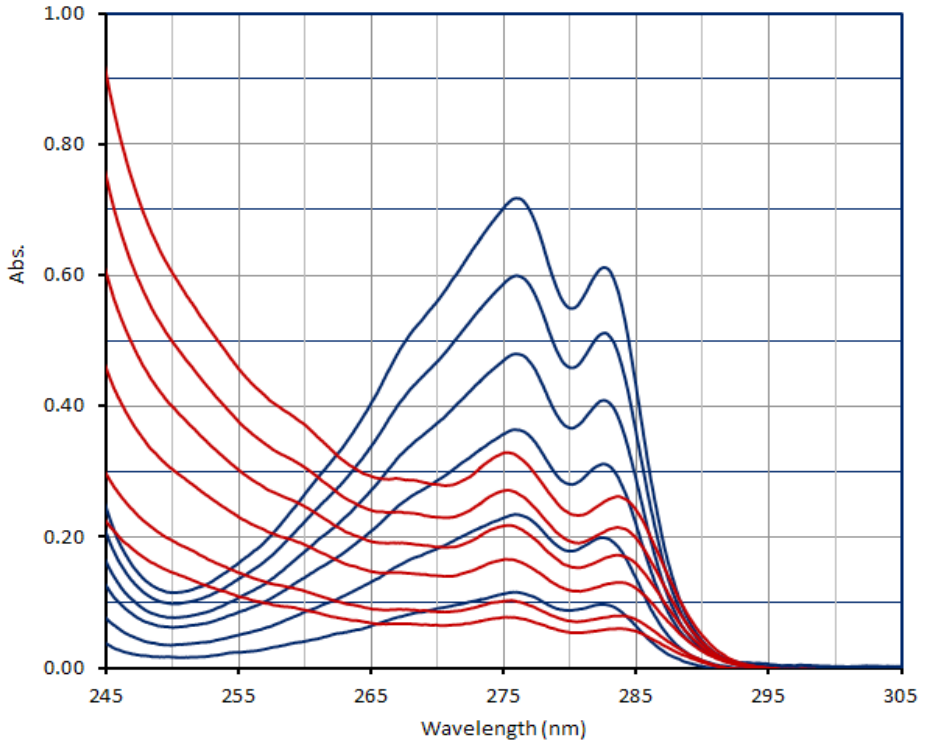

Figure 1. Original UV absorption spectra of ATE (20-120 µg/mL) (—) and CHL (15-60 µg/mL) (—) solutions in methanol.

Signal processing methods

The mathematical algorithm of the wavelet families makes this technique a suitable approach to resolve the overlapping spectral bands. In our study, we applied two different wavelet families, Coiflets with the 5 order (COIF5), and Mexican Hat (MEXH) for the analysis of overlapping spectra of ATE and CHL. After investigation of various wavelet families, it was concluded that these wavelet families were suitable to quantify ATE and CHL in their binary mixtures. The linear regression analysis of the drugs was based on the relationship between the CWT amplitudes and concentrations.

CWT spectra for ATE and CHL were obtained by plotting the CWT coefficients vs. wavelengths between 245 nm and 295 nm. COIF-CWT spectra of the drugs were indicated in Figure 2. The amplitudes at 281.6 nm and 260.0 nm were proportional to the concentration of ATE and CHL. The calibration curves for ATE and CHL were obtained by measuring the CWT amplitudes at the selected wavelengths and their statistical regression analysis results were given in Table 1.

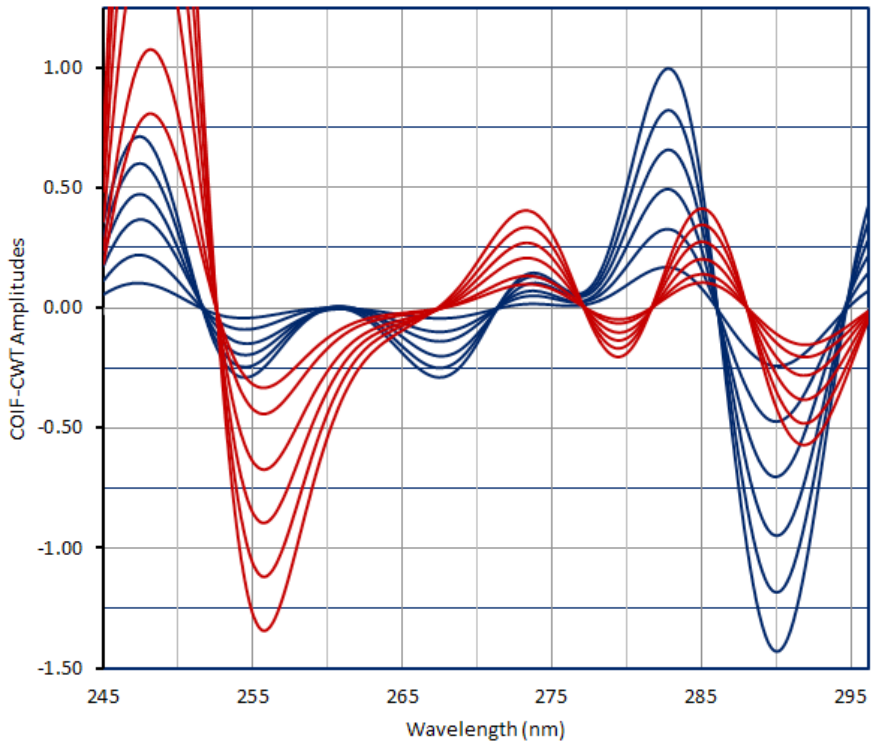

Figure 2. COIF5-CWT spectra of ATE (20-120 μg/mL) (—) and CHL (15-60 μg/mL) (—).

Table 1. Regression analysis results of ATE and CHL by COIF-CWT and MEXH-CWT methods

	COIF-CWT		MEXH-CWT	
Calibration parameters	ATE	CHL	ATE	CHL
λ (nm)	281.6	260.0	271.6	264.4
Range (μg/mL)	20-120	15-60	20-120	15-60
m	6.65×10^{-3}	-9.08×10^{-3}	2.04×10^{-2}	-1.76×10^{-2}
n	-3.44×10^{-3}	1.27×10^{-2}	3.25×10^{-3}	9.76×10^{-3}
r	0.9999	0.9997	1.0000	0.9997
SD (m)	5.13×10^{-5}	1.19×10^{-4}	4.86×10^{-5}	2.08×10^{-4}
SD (n)	4.00×10^{-3}	4.67×10^{-3}	3.78×10^{-3}	8.14×10^{-3}
SD (r)	4.30×10^{-3}	4.65×10^{-3}	4.06×10^{-3}	8.10×10^{-3}
LOD (μg/mL)	4.42	3.78	1.36	3.41
LOQ (μg/mL)	14.73	12.61	4.55	11.36

m = Slope of the linear regression equation
n = Intercept of the linear regression equation
r = Correlation coefficient of the linear regression equation
SD (m) = Standard deviation of slope
SD (n) = Standard deviation of intercept
SD (r) = Standard deviation of correlation
LOD = Limit of detection
LOQ = Limit of quantitation

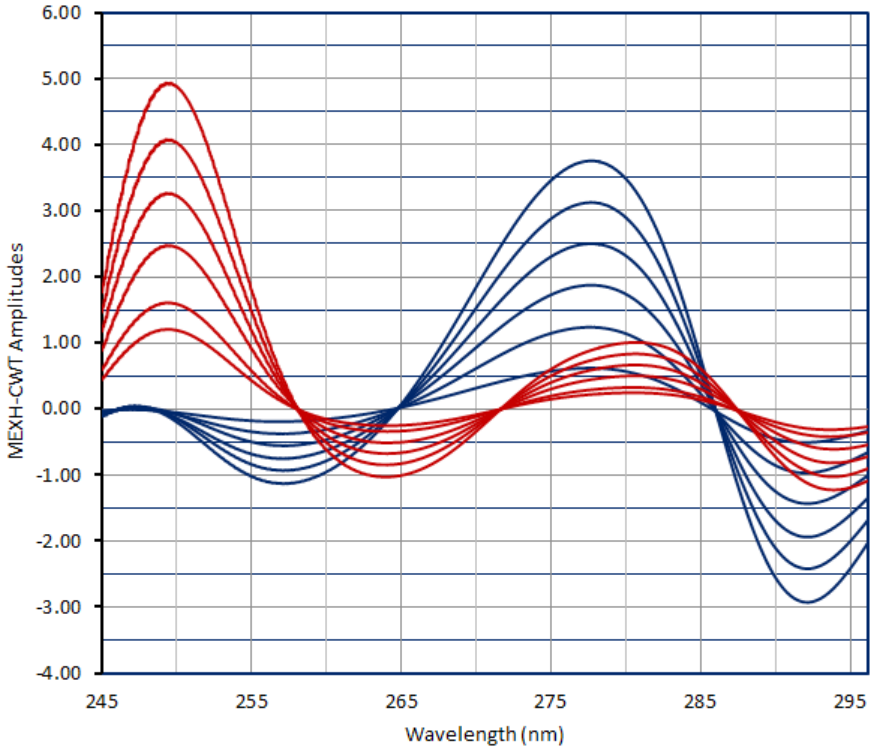

Figure 3. MEXH-CWT spectra of ATE (20-120 µg/mL) (—) and CHL (15-60 µg/mL) (—).

A similar process was repeated for the application of MEXH family. As seen in Figure 3, the suitable amplitudes were at 271.6 nm for ATE and 264.4 nm for CHL. The calibration parameters and regression analysis results were summarized in Table 1. The quantitative analysis of the subjected drugs in the analyzed samples was performed using the calculated calibration data.

Table 2. Recovery data of ATE and CHL in binary synthetic mixtures by COIF-CWT and MEXH-CWT methods

		COIF-CWT				MEXH-CWT			
Added (µg/mL)		Found (µg/mL)		Recovery (%)		Found (µg/mL)		Recovery (%)	
ATE	CHL	ATE	CHL	ATE	CHL	ATE	CHL	ATE	CHL
100	15	98.81	15.17	98.8	101.1	99.30	15.72	99.3	104.8
100	20	100.32	20.68	100.3	103.4	101.65	20.84	101.7	104.2
100	30	99.80	31.45	99.8	104.8	100.61	30.97	100.6	103.2
100	40	100.04	40.53	100.0	101.3	100.98	42.30	101.0	105.7
100	50	99.56	50.53	99.6	101.1	100.08	52.22	100.1	104.4
100	60	101.96	61.44	102.0	102.4	101.53	62.12	101.5	103.5
20	25	21.02	25.94	105.1	103.8	20.78	25.46	103.9	101.8
40	25	41.28	26.41	103.2	105.6	39.70	25.49	99.3	101.9
60	25	60.64	25.59	101.1	102.4	60.20	26.21	100.3	104.8
80	25	80.04	25.48	100.1	101.9	80.13	26.17	100.2	104.7
100	25	98.89	25.01	98.9	100.1	100.15	25.44	100.1	101.8
120	25	118.26	25.03	98.6	100.1	119.53	25.97	99.6	103.9
			Mean	100.6	102.3			100.6	103.7
			SD	1.87	1.70			1.23	1.26
			RSD	1.85	1.66			1.23	1.21

SD = Standard deviation
RSD = Relative standard deviation

Validation studies

A good linearity was obtained in the concentration range between 20–120 μg/mL for ATE and 15-60 μg/mL for CHL which was proven by the numerical values of the correlation coefficients (see Table 1). The precision of COIF-CWT and MEXH-CWT methods were reported to be successful as can be seen by the recovery, standard deviation and relative standard deviation results obtained by the analysis of 12 synthetic mixtures (see Table 2). We concluded that both wavelet families exhibited good mean recovery values and relative standard deviations.

From the standard deviation of the intercept and slope of the calibration equations, the limit of detection and the limit of quantitation were calculated and given in Table 1. Three different concentration levels (20, 60 and 120 μg/mL for ATE and 15, 40 and 60 μg/mL for CHL) were used for the intra-day and inter-day studies. This experiment was repeated six times for each concentration level. Their analysis results with recovery and relative standard deviation values, obtained by CWT signal processing methods, were given in Table 3. As can be seen in Table 3, a good accuracy and precision were reported for intra-day and inter-day studies.

Table 3. Analysis results of ATE and CHL in intra-day and inter-day samples by COIF-CWT and MEXH-CWT methods

			COIF-CWT						MEXH-CWT					
	Added (μg/mL)		Found (μg/mL)		Recovery (%)		RDS (%)		Found (μg/mL)		Recovery (%)		RDS (%)	
	ATE	CHL	ATE	CHL	ATE	CHL	ATE	CHL	ATE	CHL	ATE	CHL	ATE	CHL
Intra-day	20	15	21.38	15.78	106.9	105.2	4.47	5.57	20.53	15.99	102.6	106.6	1.34	1.73
	60	40	61.05	41.85	101.7	104.6	1.28	1.97	60.04	40.65	100.1	101.6	0.39	0.39
	120	60	118.93	60.74	99.1	101.2	1.10	3.01	118.36	61.45	98.6	102.4	0.30	1.90
Inter-day	20	15	20.84	15.37	104.2	102.5	3.22	4.51	20.67	15.51	103.3	103.4	2.93	2.66
	60	40	60.49	41.91	100.8	104.8	1.64	1.14	60.88	40.44	101.5	101.1	0.78	1.48
	120	60	120.78	61.35	100.7	102.3	0.93	1.58	120.26	60.38	100.2	100.6	1.11	2.44

RSD = Relative standard deviation

Analysis of tablet samples

Two different CWT methods based on the use of UV spectra were used for the simultaneous quantitation of ATE and CHL in a solid dosage form. The assay results with mean values, standard deviations and relative standard deviations were summarized in Table 4. Both methods gave successful results for the quality control of the tablets containing ATE and CHL.

Table 4. Experimental results of ATE and CHL in tablet samples by COIF-CWT and MEXH-CWT methods.

	mg/tablet			
Sample number	COIF-CWT		MEXH-CWT	
	ATE	CHL	ATE	CHL
1	99.7	25.2	95.8	25.8
2	98.1	25.2	99.9	25.5
3	98.8	25.0	98.3	26.7
4	98.8	25.7	98.3	26.7
5	100.4	25.0	98.1	26.4
6	99.7	25.5	99.9	25.8
7	99	24.5	97.2	25.7
8	98.3	25.4	97.4	25.5
9	99.4	25.4	97.7	25.0
10	99	25.5	97.8	24.9
Mean	99.1	25.2	98.0	25.8
SD	0.69	0.35	1.22	0.61
RSD	0.70	1.37	1.24	2.38

SD = Standard deviation
RSD = Relative standard deviation

Conclusion

This work presents new CWT-based analytical methods for the simultaneous quantitative estimation of ATE and CHL in tablet preparations. The CWT analysis were performed without the use of chemical pre-treatment or preliminary separation step. We concluded that both COIF and MEXH wavelet families showed good recovery and relative standard deviations values in the artificial mixtures. The proposed methods were applied directly to the original UV spectra of the samples. We think that both signal processing methods are promising approaches for the quality control and routine analysis of commercial tablets containing ATE and CHL.

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