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Continuous Wavelet Transform Methods for the Simultaneous Quantification of Brimonidine Tartrate and Timolol Maleate in an Eye Drop Formulation

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Abstract

In this research paper, the UV spectral data vectors of samples were processed by two different continuous wavelet transform (CWT) approaches for simultaneous quantification of Brimonidine tartrate (BRI) and timolol maleate (TIM) in an eye drop formulation. The application of the CWT methods to the analysis of BRI-TIM mixtures did not require the use of a preliminary chemical separation. After preliminary experiments, Symlet5 continuous wavelet transforms (sym5-CWT) and Mexican Hat continuous wavelet transform (mexh-CWT) were found to be very suitable to simultaneously quantify the analyzed drugs. Calibration curves for BRI and TIM in the concentration range of 5.0-25.0 μg/mL and 8.0-72.0 μg/mL were obtained by measuring the CWT amplitudes in the wavelet domain. The proposed sym5-CWT and mexh-CWT methods were validated and applied to the quantitative analysis of commercial eye drop samples containing the BRI and TIM drugs.

Keywords: Continuous Wavelet Transform; UV-VIS spectrophotometry; Quantification; Brimonidine tartrate; Timolol maleate

Introduction

Numerous methods are available to analyze chemical components. Particularly, high-performance liquid chromatography (HPLC) (or ultra-performance liquid chromatography (UPLC)) and spectrophotometry are two of the most commonly used in analytical chemistry. In the analysis of pharmaceuticals, HPLC or UPLC is a standard techniques used in related labs. In HPLC analyses, one of the main problems is to find optimal chromatographic conditions e.g. flow rate, column temperature, type and pH of inorganic modifiers in mobile phase. In some cases, this requires long laboratory works and excessive consumption of mobile phase to get suitable chromatographic conditions giving a perfect separation or exact elution of drugs. Despite its advantages, HPLC can be costly due to the mentioned challenges. Additionally, the chromatographic technique may not always give expected separation and quantification results.

Visible-ultraviolet (VIS-UV) spectrophotometry is another instrumentation method for the analysis of drug substances. However, the determination of drug substances in mixtures containing two or more drugs is not possible by direct absorbance measurements due to

overlapping spectral bands. In order to overcome the mentioned disadvantages of HPLC (or UPLC) and UV-VIS spectrophotometry, scientists require to develop or implement new methods for the analysis of active compounds in complex samples. In this regard, CWT approach is a powerful signal-processing tool for data reduction, de-noising, baseline correction and resolution of overlapping spectra of analyzed drugs [1].

In our previous works, CWT methods with zero-crossing technique and ratio spectra procedure were directly applied to the simultaneous quantitative resolution of binary and ternary mixtures [2] and successful results were obtained.

Brimonidine tartrate (BRI) is an effective ocular hypotensive agent which is currently undergoing clinical evaluation [3]. Timolol Maleate (TIM) is chemically described as (S)-1-(tertbutylamino)-3-[(4-morpholino-1, 2, 5-thiadiazol-3-yl) oxy]-propan-2-ol Maleate which is official in U.S.P. It was introduced for clinical use in the management of glaucoma in 1978 since then none of them ewer beta blocker have been found more effective than TIM [4].

The literature survey showed that there were many analytical methods for the quantitative analysis of BRI and TIM, e.g. HPLC [5-11], spectrophotometry [12-14], flourimetric methods [15,16], high-performance thin-layer chromatography [17], capillary electrophoresis [18], gas chromatography–mass spectrometry [19], chemiluminescence method [20], membrane electrodes method [21] and extraction-photometric method [22].

This paper describes new implementations of the sym5-CWT and mexh-CWT methods to the UV absorption spectra for the simultaneous quantification of BRI and TIM in synthetic binary mixtures and commercial ophthalmic samples. The validation of both sym5-CWT and mexh-CWT were validated by analyzing the different validation samples e.g., synthetic binary mixtures, intra-day and inter-day samples and standard addition samples. Then, then proposed wavelet transforms were successfully applied to the real samples. The results obtained by CWT methods were statistically compared with those obtained by the UPLC method developed in the literature [23] and good agreements were reported.

Experimental

Instrument and Software

The UV absorption spectra data were obtained using Shimadzu UV-2520 double beam UV–Vis spectrophotometer connected to a computer loaded with UV Probe 2.32 software. The spectra of the calibration and validation samples were plotted with the intervals of $\Delta\lambda$= 0.1 nm in wavelength range of 220.0-340.0 nm. Microsoft EXCEL and Wavelet toolbox in Mat lab software (The Math Works, Natick, MA, USA) were used for the spectral data treatments and statistical calculations.

Pharmaceutical Ophthalmic Dosage Form

A commercial pharmaceutical preparation, Combigan® eye drop (produced by ALLERGAN Ind. Pharm, Istanbul, Turkey) containing BRI (2 mg/mL) and TIM (5 mg/mL) was investigated. Brimonidine tartrate and timolol maleate were kindly obtained from World Medicine Ind. Pharm., Istanbul, Turkey. BRI and TIM standards were taken from a national pharmacy, Ankara, Turkey.

Standard, Calibration and Validation Solutions

Stock solutions of both compounds separately were prepared by dissolving 10 mg BRI and 10 mg TIM in a volume of 100 mL in methanol. The calibration samples of BRI and TIM in the concentration ranges 5.0-25.0 µg/mL and 8.0-72.0 µg/mL were prepared by diluting stock solutions. For the method validation processes, 11 different synthetic mixtures containing BRI and TIM in the working concentration ranges were prepared from the above stock solutions. Inter-day and intra-day studies containing BRI and TIM at three different concentration levels (low: 6 µg/mL, medium: 15 µg/mL and high: 24 µg/mL for BRI and low: 10 µg/mL, medium: 40 µg/mL and high: 70 µg/mL for TIM) were freshly prepared. Standard addition samples containing three different levels for BRI and TIM; low: 6 µg/mL, medium: 12 µg/mL and high: 16 µg/mL and low: 8 µg/mL, medium: 30 µg/mL and high: 40 µg/mL, were prepared, respectively. All samples were prepared in methanol during this study.

Preparation of Commercial Eye Drop Samples.

A 1.0 mL of commercial eye drop sample was transferred into a 50 mL calibrated flask and then the volume was made up to 50 mL with methanol. The content of the sample was sonicated for about 10 min and then the solution was filtered through a 0.45µm Sartorius Minisart membrane filter. The similar sample preparation was repeated 10 times for the analysis of the commercial eye drop samples.

Result and Discussion

CWT Application to the UV Spectra Data

The absorption spectra of the calibration solutions of BRI and TIM in the range of 5.0-25.0 µg/mL, 8.0-72.0 µg/mL were recorded between 220.0-340.0 nm as

indicated in Figure 1a. As it can be seen in this figure 1a, 220.0–340.0 nm overlap with each other. To overcome this problem, we applied new signal processing methods (the CWT methods) to the raw UV data vectors obtained from the UV absorption spectra of BRI and TIM for the calibration samples, synthetic mixtures, intra-day and inter-day samples, standard addition samples and commercial eye drop samples. In practice, the absorption spectra of BRI and TIM over the range of we tested several wavelet families for the find suitable signal processing approach to reach the highest recovery results of the simultaneous quantification of BRI and TIM in eye drop samples. After these experiments, we decided that two wavelet families, sym5 and mexhwavelet tools for the quantitative estimation of the analyzed drugs.

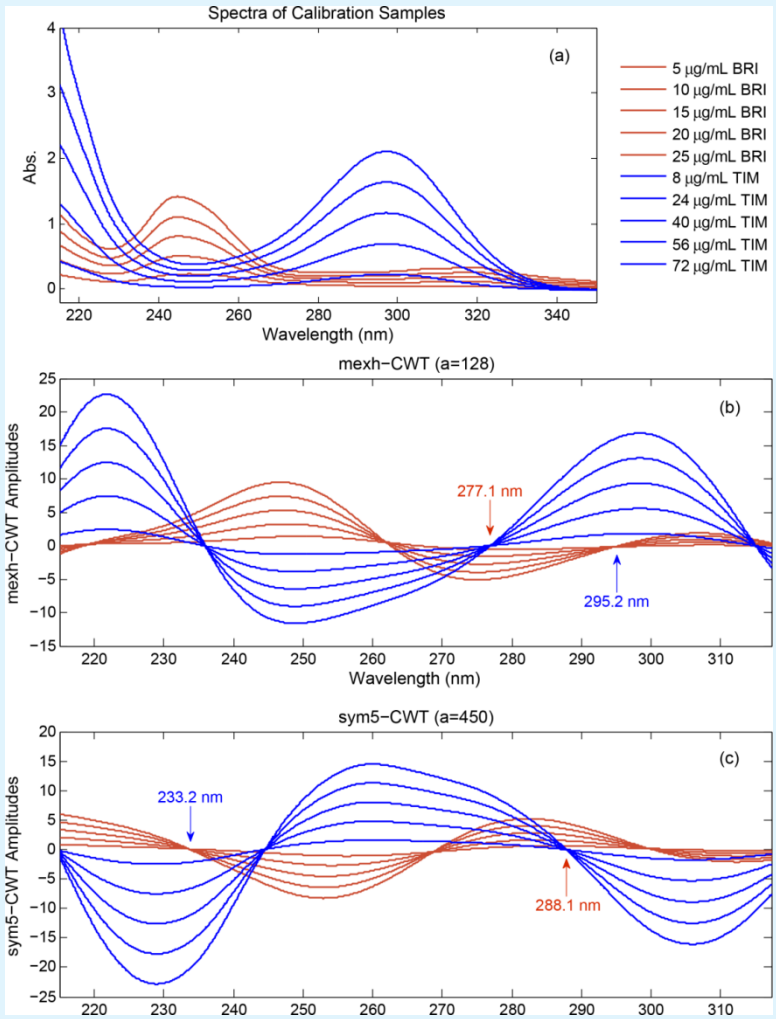

Figure 1a: UV spectra of BRI and TIM of the calibration samples, b) the mexh-CWT spectra and c) the sym5-CWTspectra.

The chosen sym5-CWT and mexh-CWT methods were applied to the UV spectral vectors of the calibration samples in the 220.0-340.0 nm wavelength regions and the sym5-CWT and Mexh-CWT spectra were obtained by plotting the CWT-coefficients against the wavelength. Figure 1b and c shows the sym5-CWT and mexh-CWT spectra, respectively. In the application of the mexh-CWT method, calibration graphs were calculated by measuring the amplitudes at a wavelength of 277.1 nm for BRI and 295.2 nm for TIM. Calibration graph in the application of the sym5-CWT method; was computed by using the measurements of the amplitudes at 233.2 nm for BRI and 288.1 nm for TIM. The linear regression analysis of calibration samples and corresponding statistical results were illustrated in Table 1. Figure 2 shows the calibration lines for BRI and TIM using the proposed signal processing methods. BRI and TIM in the analyzed samples were determined by the calibration curves of the related drugs.

Parameter	mexh-CWT		sym5-CWT	
	BRI	TIM	BRI	TIM
m	0.2228	0.2231	0.1924	0.2815
n	0.5745	-0.0348	-0.4097	0.1067
r	0.9997	1	0.9998	1
SD (m)	$3.35x10^{-3}$	$3.50 x10^{-4}$	$2.23 x10^{-3}$	$4.23 x10^{-4}$
SD (n)	$5.56 x10^{-2}$	$5.56 x10^{-2}$	$3.69 x10^{-2}$	$3.69 x10^{-2}$
SD (r)	$5.30 x10^{-2}$	$1.77 x10^{-2}$	$3.52 x10^{-2}$	$2.14 x10^{-2}$
LOD	0.29	1.39	0.27	0.55
LOQ	0.97	4.62	0.9	1.82

Table 1. Linear regression analysis and statistical results of mexh-CWT and sym5-CWT methods.

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 error of slope
SD (n) = Standard error of intercept
SD(r) = Standard error of correlation
LOD = Limit of detection (µg/mL)
LOQ = Limit of quantitation (µg/mL)

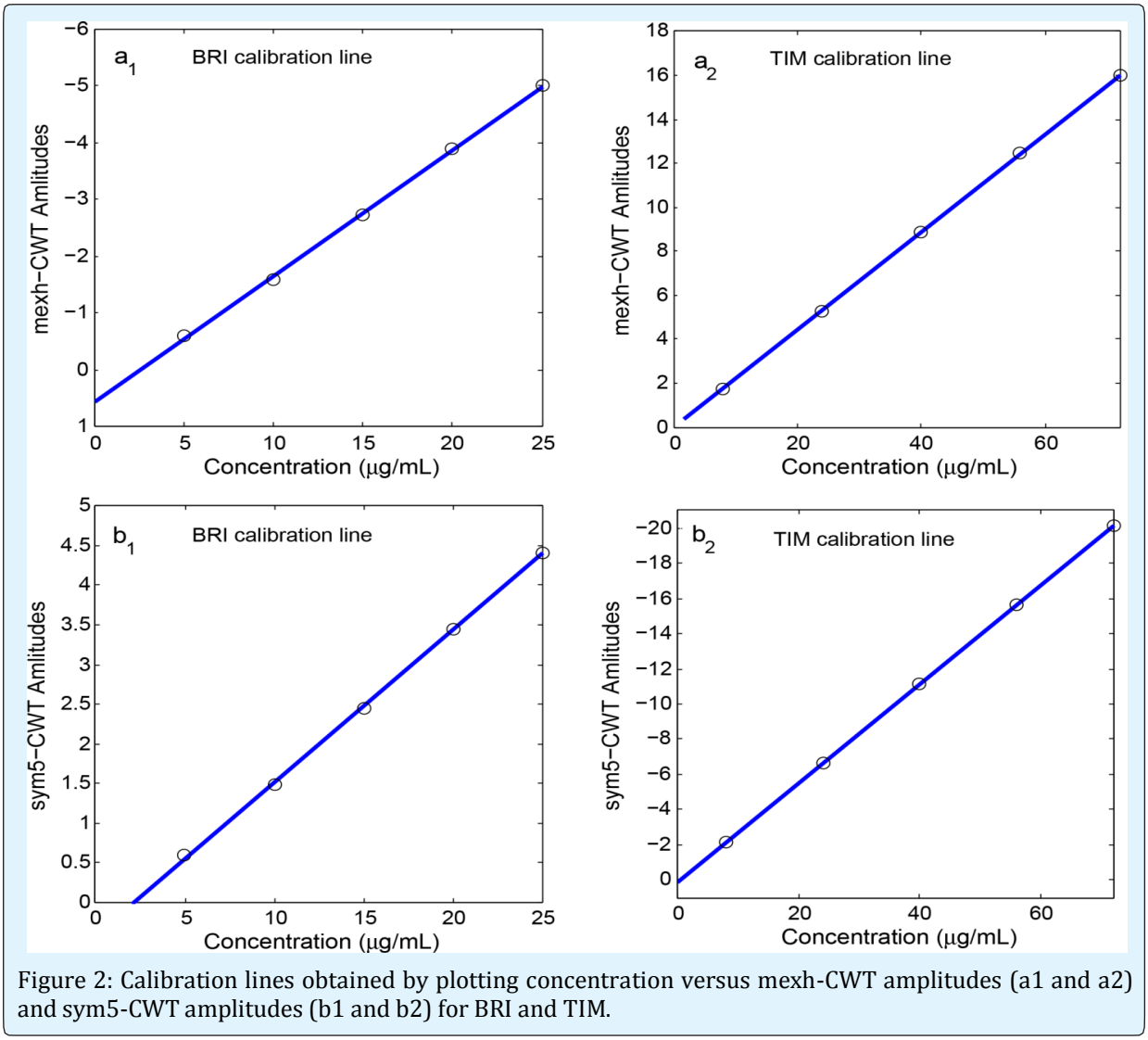

Figure 2: Calibration lines obtained by plotting concentration versus mexh-CWT amplitudes (a1 and a2) and sym5-CWT amplitudes (b1 and b2) for BRI and TIM.

Validation of the Applied CWT Methods

In the mexh-CWT and sym5-CWT applications, the a good linearity for BRI and TIM in the working range of 5.0-25.0 µg/mL an 8.0-72.0 µg/mL, respectively, was reported from the regression coefficients indicated in Table 1. The limit of quantitation (LOD, signal/noise=3) and limit of detection (LOQ, signal/noise=10) were computed using standard deviation of the linear regression equations of the analyzed drugs.

For the validity of 11 different synthetic mixtures were prepared and analyzed by the mexh-CWT and Sym5-CWT methods. Their recovery results were listed in Table 2.The analysis results demonstrate that satisfactory accuracy and precision of the proposed CWT signal processing methods. In order to visualize the reproducibility of the Mexh-CWT and Sym5-CWT methods, the intra-day and inter-day experiments were done by analyzing the intra-day and inter-day samples as described in the section "2.3. Standard, calibration and validation solutions". Percent mean recoveries; relative standard deviations and relative standard errors were presented in Table 3. These results show that the newly applied CWT methods are very useful for the analysis of BRI and TIM in samples with desirable precision and accuracy. Before analyzing commercial eye drop samples, the standard addition technique was used to determine whether the excipients in the commercial eye drop preparation were an effect on the analysis of the mexh-CWT and Sym5-CWT methods. In order to observe the presence and absence of the excipient effect on the analysis of the related drugs, standard addition samples were analyzed and then assay results were presented in Table 4. From analysis results in Table 4, noexcipient effect on the determination of BRI and TIM was reported.

	Added (µg/mL)		Mexh-SDD				Sym5-SDD			
			Found (µg/mL)		Recovery (%)		Found (µg/µL)		Recovery (%)	
Kod	BRI	TIM	BRI	TIM	BRI	TIM	BRI	TIM	BRI	TIM
M1	5	40	5.1	39.7	101.9	99.2	4.9	39.8	97.9	99.4
M2	10	40	10.6	39.5	105.6	98.8	10.1	39.9	101.4	99.8
M3	15	40	15.4	39.4	102.5	98.5	14.9	39.8	99.6	99.5
M4	20	40	20.4	39.6	101.9	99	19.9	40.1	99.5	100.2
M5	25	40	24.9	39.7	99.7	99.3	24.5	40.5	98.1	101.1
M6	16	8	16.7	7.8	104.3	97.3	16.6	8.2	103.5	102.4
M7	16	24	16.4	23.6	102.4	98.5	16.1	24.4	100.9	101.8
M8	16	40	16.4	39.6	102.6	99.1	16	40.2	100.1	100.4
M9	16	56	16	55.3	100	98.8	15.6	55.8	97.4	99.7
M10	16	72	16.7	71.8	104.5	99.7	16.1	73.5	100.8	102
M11	20	50	20.4	49.8	102.2	99.6	19.8	50.3	99.2	100.6
				$\bar{X}$	102.5	98.9		$\bar{X}$	99.8	100.6
				SD	1.78	0.64		SS	1.76	1.06
				RSD	1.73	0.65		BSS	1.77	1.05

Table 2: Analysis results of applying Mexh-CWT and Sym5-CWT methods to BRI-TIM binary synthetic mixtures.

$\bar{X}$= Mean
SD= Standard deviation
RSD= Relative standard deviation

	Added (µg/mL)		Found (µg/mL)			
			mexh-CWT		sym5-CWT	
	BRI	TIM	BRI	TIM	BRI	TIM
Inter-day	6	10	6.1	9.55	6.06	10.46
	15	40	15.26	39.60	14.91	40.06
	24	70	24.12	70.20	23.53	71.95
Intra-day	6	10	5.56	9.55	5.94	10.44
	15	40	15.12	39.82	14.87	40.3
	24	70	24.1	70.72	23.57	72.52
			Recovery (%)			
			mexh-CWT		sym5-CWT	
			BRI	TIM	BRI	TIM

Inter-day			101.6	95.5	101.1	104.6
			101.7	99.0	99.4	100.2
			100.5	100.3	98	102.8
Intra-day			92.6	95.5	99	104.4
			100.8	99.5	99.1	100.8
			100.4	101.0	98.2	103.6
			RSD (%)			
			mexh-CWT		sym5-CWT	
			BRI	TIM	BRI	TIM
Inter-day			0.96	0.21	0.68	0.3
			0.68	0.13	0.36	0.2
			0.24	0.21	0.1	0.32
Intra-day			5.52	0.11	0.21	0.07
			0.09	0.20	0.04	0.20
			0.11	0.26	0.23	0.41
			RSE (%)			
			mexh-CWT		sym5-CWT	
			BRI	TIM	BRI	TIM
Inter-day			1.63	-4.51	1.06	4.61
			1.72	-1.00	-0.57	0.15
			0.52	0.28	-1.97	2.79
Intra-day			-7.36	-4.47	-0.98	4.39
			0.82	-0.46	-0.87	0.75
			0.42	1.03	-1.78	3.60

Table 3: Analysis results of intra-day and inter-day samples.

RSD= Relative standard deviation
RSE= Relative standard error (n= 3 for every concentration level)

Added (µg/mL)			Found (µg/mL)			
mexh-CWT			sym5-CWT			
BRI	TIM	BRI	TIM		BRI	TIM
Formulation	6	8	5.87	8.03	5.95	8.17
Formulation	12	30	12.04	30.16	12.12	30.76
Formulation	16	40	16.07	41.03	16.41	41.63
Recovery (%)						
mexh-CWT			sym5-CWT			
BRI		TIM	BRI		TIM	
97.9		100.4	99.2		102.1	
100.3		100.5	101.0		102.5	
100.4		102.6	102.6		104.1	
RSD (%)						
mexh-CWT			sym5-CWT			
BRI		TIM	BRI		TIM	
0.18		0.23	0.15		0.20	
0.23		0.83	0.10		1.60	
0.21		0.28	0.68		0.90	

Table 4: Assay results of standard adding samples.

RSD= Relative standard deviation

Analysis of Commercial Eye Drop Formulation

In this research paper, the proposed signal processing methods, Mexh-CWT and Sym5-CWT, were applied to the simultaneous quantitative quantification of BRI and TIM in eye drop samples. Assay results were presented in Table 5. As it can be seen in Table 5, a good accordance with the acceptable standard deviation and relative standard deviation was reported for the analysis results obtained by applying the validated mexh-CWT and sym5-CWTmethods to the commercial eye drop formulation. Using one-way ANOVA test, the determination results of BRI and TIM in analyzed commercial sample were statistically compared with those of UPLC method in the literature [23]. It was observed that there is no difference between analysis by signal processing methods and UPLC approach.

mg/mL						
	mexh-CWT		sym5-CWT		*UPLC method	
Exp. No.	BRI	TIM	BRI	TIM	BRI	TIM
1	2.01	4.98	1.99	5.02	1.94	5.06
2	1.99	5.02	2.02	5.01	1.94	5.01
3	1.94	5.06	1.94	5.08	1.98	4.97
4	2.01	5.04	1.96	4.98	1.94	4.93
5	1.97	5.12	2.03	4.99	2	5.15
6	2	5.03	2.01	4.97	2.03	5.08
7	2.04	5.05	2.04	4.98	2.05	5.29
8	1.95	5.03	1.98	4.96	2.06	5.17
9	1.96	4.92	1.97	4.95	1.99	5.01
10	1.98	5.01	1.98	4.91	1.96	4.96
Mean	1.98	5.03	1.99	4.99	1.99	5.06
SD	0.03	0.05	0.03	0.05	0.05	0.11
RSD	1.51	1.05	1.71	0.91	2.37	2.23
Confidence interval	Mean ± 0.02	Mean ± 0.04	Mean ± 0.02	Mean ± 0.04	Mean ± 0.04	Mean ± 0.08

Table 5: Analysis results of the commercial eye drop formulation obtained using the Mech-CWT and Sym5-CWT methods. (Lable claim: BRI 2.0 mg/mL TIM 5.0 mg/mL).

SD= Standard deviation
RSD= Relative standard deviation
*literature method

	Experiment Number		**Sum**		**Average**		**Variance**					
Methods	BRI	TIM	BRI	TIM	BRI	TIM	BRI	TIM				
UPLC	10	10	19.89	50.63	1.99	5.06	0.0021	0.0126				
Mexh-SDD	10	10	19.9	50.03	1.99	5	0.0014	0.0078				
Sym5-SDD	10	10	19.92	49.85	1.99	4.99	0.001	0.0021				
	SS		df		MS		F-calculated		P-value		F-table	
Variance	BRI	TIM	BRI	TIM	BRI	TIM	BRI	TIM	BRI	TIM	BRI	TIM
Between methods	0.0132	0.0565	4	4	0.0033	0.0141	1.93	2.32	0.12	0.07	2.58	2.58
Within methods	0.0767	0.2737	45	45	0.0017	0.0061						
Total	0.0899	0.3302	49	49								

Table 6: ANOVA test of mexh-CWT, sym5-CWT method and literature method (UPLC).

SS= Sum of squares
df = Degrees of freedom
MS = Mean of squares

Conclusion

In this manuscript, a new combined use of CWT approaches was applied to the quantitative resolution of the overlapping UV spectral bands for the simultaneous quantitative analysis of BRI and TIM in an eye drop formulation. The simultaneous quantification of BRI and TIM in an eye drop formulation by were carried out without any chemical pre-treatment or any preliminary separation step. Compared to the UPLC method [23], the comparable assay results were obtained by applying mexh-CWT and Sym5-CWTmethods to the quantitation of the related active substances in the analyzed samples. The assay results indicate that these CWT approaches are very useful for the quality control and a routine analysis of the commercial eye drop formulation containing BRI and TIM drugs.

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