CONTINUOUS WAVELET TRANSFORM FOR SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND ROSUVASTATIN IN COMBINED PHARMACEUTICAL FORMULATIONS

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The combination of continuous wavelet transforms (CWT) with zero-crossing strategy and spectral ratio treatment was described for simultaneous determination of atorvastatin (AT) and rosuvastatin (RVS). Meyer-CWT and Gaus8-CWT of wavelet families were found to be appropriate for spectral analyses of original overlapping signals. Also, it was established that Morl-CWT and fk8-CWT were applicable to signal analysis of the ratio spectra of AT/RVS and RVS/AT. The calibration graphs of CWT methods for AT and RVS were obtained from the working concentration ranges of 5–25 and 10–30 μg/mL, respectively. The applicability of proposed signal processing methods was successfully tested for simultaneous detection of the AT and RVS in combination dosage formulations (without a primary separation). The CWT approach also stayed within good analytical parameters (precision, accuracy, and detection limit) for the management of quality and routine analyses of both drugs in comparison with derivative procedure. The results of proposed method were in agreement with those obtained from HPLC.

***Keywords*:** *atorvastatin, rosuvastatin, continuous wavelet transform, signal processing.*

МЕТОД НЕПРЕРЫВНОГО ВЕЙВЛЕТ-ПРЕОБРАЗОВАНИЯ ДЛЯ ОДНОВРЕМЕННОГО ОПРЕДЕЛЕНИЯ АТОРВАСТАТИНА И РОЗУВАСТАТИНА В КОМБИНИРОВАННЫХ ФАРМАЦЕВТИЧЕСКИХ ПРЕПАРАТАХ

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Описана комбинация непрерывного вейвлет-преобразования (CWT) с переходом через 0 и обработкой спектральных соотношений для одновременного определения аторвастатина (AT) и розувастатина (RVS). Установлено, что вейвлет-семейства meyer-CWT и Gaus8-CWT подходят для спектрального анализа исходных перекрывающихся сигналов, Morl-CWT и fk8-CWT применимы для анализа сигналов спектров отношений AT/RVS и RVS/AT. Калибровочные кривые для AT и RVS получены для рабочих диапазонов концентраций 5–25 и 10–30 мкг/мл. Применимость предложенных методов обработки сигналов успешно протестирована для одновременного обнаружения AT и RVS в комбинированных дозированных препаратах (без первичного разделения). Подход CWT также имеет хорошие аналитические параметры (прецизионность, точность и предел обнаружения) для регулирования качества и состава обоих препаратов. Результаты предложенного метода согласуются с полученными с помощью высокоэффективной жидкостной хроматографии.

Ключевые слова: *аторвастатин, розувастатин, непрерывное вейвлет-преобразование, обработка сигналов.*

Introduction. Atorvastatin (AT) and rosuvastatin (RVS) are members of the statin class of medicines utilized for treating hypercholesterolemia in patients suffering from a diagnosed cardiovascular illness and those at higher risks for developing atherosclerosis. The mentioned medicines suppress the rate limiting major enzyme which is called 3-hydroxy-3-methyl-glutarylcoenzyme A (HMG-CoA) reductase and contributes to biosynthesize cholesterol. Before approving RVS in 2003, AT has been a medicine with the highest efficiency of statin class [1]; however, recent researchers established RVS as one of the potential inhibitors of HMG-CoA reductase, which applies a greater LDL (low density lipoprotein) declining impact than that of the remaining statins [2, 3]. In addition to lipid declining impacts and cholesterol reduction, the statins contribute to some other potentially independent roles such as anti-oxidative [4–6], antitumor [7], anti-inflammatory [8, 9], immunomodulator [5, 10], anti-malarial, and bone forming agents [11, 12]. In some cases, a statin is combined and administered with a heart medication or other statins. Therefore, the development of methods for determination of statins is significant. Also, the simultaneous determination of statins has some benefits in combination therapy.

The authors used the procedures to simultaneously detect the AT and RVS together or in combination with other drugs in pharmaceutical and biological samples [13–17]. The reported analytical methods require steps like extraction, derivatization, and separation in the course of analyses, which cost a lot and are time-consuming. Therefore, is a need to develop analytical procedures to overcome and eliminate these disadvantages and problems. Furthermore, the simultaneous analyses of combined drug formulations are important matters for analytical chemistry due to interfering spectral bands or unresolved peaks of the compositions.

The derivative spectrophotometric technique has been applied for quantifying the compounds with overlapping peaks. It is simple and rapid, but this technique has multiple drawbacks to the original absorption of spectra, including decreasing the peak intensity in higher-order derivations, requiring further smooth function modes and extra scaling factor procedures. Therefore, the derivative method cannot sometimes be used in the simultaneous quantitative analysis purposes [18–20].

In recent years, experts in the field of signal processing utilized wavelet transform (WT) techniques for enhancing potential power of various spectral techniques. It is notable that WT greatly helps the simultaneous detection of analytes while studying spectral analyses. It is actually one of the proper signal processing tools of denoising, correcting the base-line, and resolving the multi-component overlapping spectra. According to the findings, a popular technique like continuous wavelet transform (CWT) in combination with the zero-crossing technique and ratio spectra (RS) treatment would give better outputs in simultaneous determination of the multi-component of different samples [21–23]. CWT provides a higher S/N in comparison with the classical derivative technique utilizing the zero-crossing technique, and it also has varied wavelet families to resolve the overlapping spectra [24, 25].

The main purpose of this investigation is to develop a simple, sensitive, precise, and affordable analytical method for simultaneous detection of AT and RVS without a need for a separate and distinct method in the pure forms or their mixed formulations based on the combined CWT and zero-crossing strategy and RS treatment. Therefore, we examined diverse wavelet families within various scale parameters. Hence, satisfactory results were obtained for quantitatively resolving and analyzing the target drugs.

Theoretical background (wavelet transform). According to the research papers, WT is a robust technique for processing the signals in chemistry as well as the other fields in science. In contrast to traditional Fourier transform, WT decomposes signal in the time-frequency domain, and it then provides many advantages over the conventional frequency decomposition in the analysis of spectral signals. In fact, the wavelet has been described as the number of scaled and dilated functions $\psi_{a,b}(\lambda)$ that is a derivation of a basic mother wavelet $\psi(\lambda)$:

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

where parameter *a* stand for the scale parameter, which is a positive variable and is utilized to control the scaling, and parameter *b* indicates the translation factor employed to shift it. For different scaling values of parameter *a*, CWT provides wavelet coefficients versus parameter *b* [26]. We show the capability of CWT to extract data for the simultaneous detection of AT and RVS in a combined formulation. Various wavelet families including Meyer, Gaussian (gaus), Fejer korovkin (fk), Morlet (morl), Coiflet (coif), Daubechies (db), Haar, Symlet (sym), and Mexican hat (mexh) were studied.

Experimental. According to the research design, UV spectra were measured by a Shimadzu UV-160A double-beam spectrophotometer. The absorption spectra were recorded in the range from 200 to 350 nm. Subsequently, the conventional and ratio absorption spectra modes were processed. The spectral data processing and WT procedures have been run by means of the wavelet toolbox in Matlab software 9.3 (R2017b). The various scaling parameters (a) must be used to obtain the optimum spectral resolution. The zero-crossing point detection was applied to data analysis for the purpose of simultaneous determination of AT and RVS. The zero-crossing points of each component of the mixture are the only functions of the others. Finally, the statistical calculations have been performed using MS Excel.

The standard stock solutions of AT and RVS have been procured via dissolving 10 mg of drugs in 100 mL of methanol (100 μg/mL). Calibration solutions of AT and RVS in the ranges of 5–25 and 10–30 μg/mL were prepared, respectively. The singlet and the mixtures of two statins were modeled and administered by the above procedure. Commercial pharmaceutical tablets including Lipitor (AT 20 mg) and Crestor (RVS 20 mg) were studied.

High-performance liquid chromatography (HPLC) as implemented on a Knauer Autosampler 3950 (AZURA) with diode array detector at the room temperature. A Phenomenex C18 (150×4.6 mm, 5 μm) column was used. The chromatographic separation was performed using isocratic elution with methanol–water (68:32, v/v; pH adjusted to 3.0) [14].

Results and discussion. According to the analyses, the absorption spectra of the standard solution of AT and RVS in concentration levels of 5–25 and 10–30 μg/mL, respectively, were obtained ranging from 200 to 350 nm. Figure 1 depicts the overlapping absorption spectra of AT and RVS. It shows that the UV spectrophotometric method cannot be used for simultaneous quantitative analyses of AT and RVS as the combined form. For this purpose, the related overlapping spectra have been processed and resolved by the means of CWT. CWT methods in combination with the zero-crossing procedure (the absorbance signals and the ratio treatment of the signals) served for simultaneous quantification of AT and RVS. Furthermore, the first derivative spectrophotometry (DS_1) was also followed for comparing outputs yielded by two techniques (CWT and DS_1). These signal processing techniques are rapid, precise, and accurate in economic analysis without a need for a separation stage.

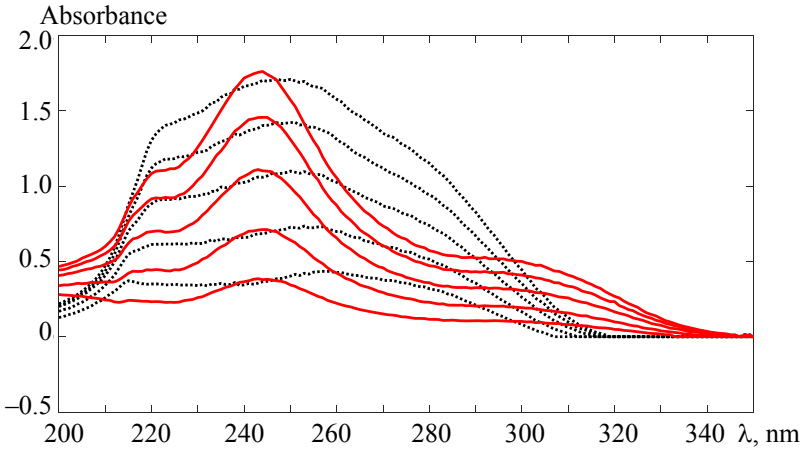

Fig. 1. The absorption spectra of atorvastatin (dotted line): 5, 10, 15, 20, and 25 μg/mL, and rosuvastatin (solid line): 10, 15, 20, 25, and 30 μg/mL.

CWT method. The one-dimensional (1D) data vectors of the UV spectrum of the standard calibration solutions for the two drugs (Fig. 1) and their mixture formulations were inserted into MS Excel software. Then we used the wavelet toolbox in Matlab to process these vectors. Various families of wavelet transform in several dilation variables (a) have been investigated to obtain optimum CWT technique for providing the most acceptable wavelet with regard to the zero-crossing points, the ratio of the signal to the noise, and the recovery for the simultaneous determination of AT and RVS. It was established that Meyer-CWT (a = 16) and gaus8-CWT (a = 32) offered the optimum signal transformation of the real UV spectra. Therefore, the above-mentioned CWTs were applied to the analysis of both compounds. Moreover, CWT-coefficients have been plotted against the wavelengths by Meyer-CWT and gaus8-CWT spectra (Fig. 2). Furthermore, we

used linear regression analysis for determining AT and RVS concentrations and obtained the calibration graphs for the two medicines. Such a condition has been implemented on the basis of a correlation between CWT-amplitude and the concentration on zero-crossing points. According to Fig. 2, 338 nm (AT) and 304 nm (RVS) for the Meyer-CWT method, and also 318 nm (AT) and 278 nm (RVS) for the gaus8-CWT method were found as zero-crossing points. Hence, we made a comparison between the presented procedures to analyze the medicines in a pure powder. The results of analytical parameters were presented in Table 1. According to the table, low LODs and LOQs and also high correlations were observed by the Meyer-CWT and Gaus-CWT methods. Moreover, the standard errors of calibration dependences (slope, intercept, and correlation) were small, so that they can also justify the choice of the mentioned CWTs. These values were comparable to those of the other CWT techniques.

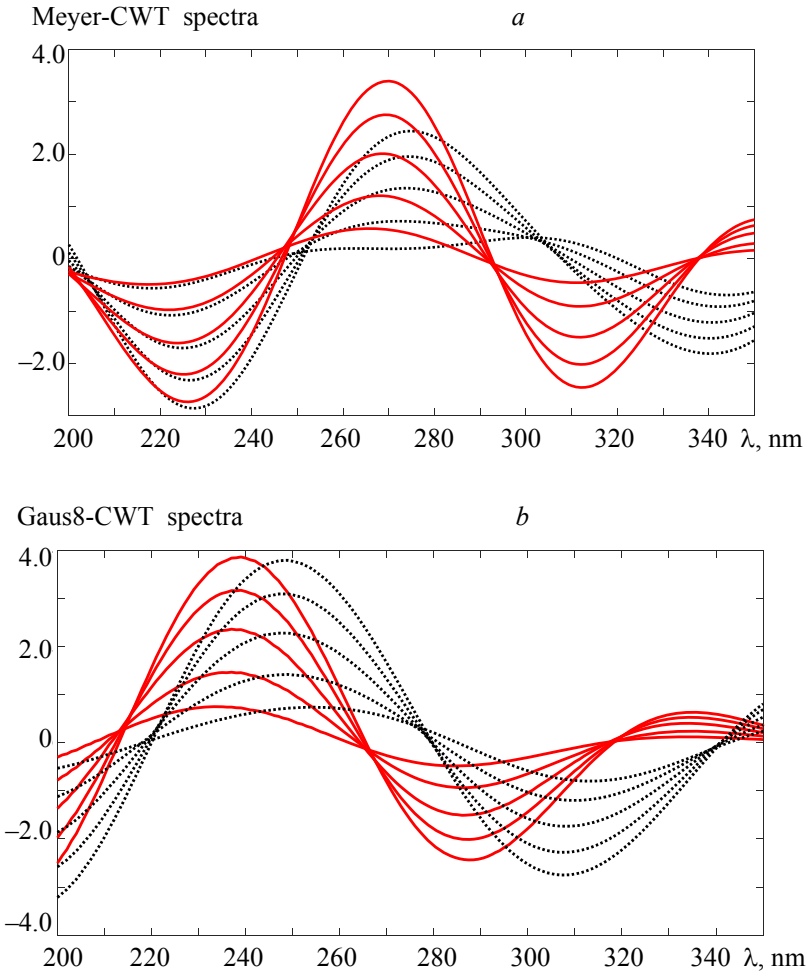

Fig. 2. The Meyer-continuous (a) and the gaussian8-continuous (b) wavelet transform spectra of atorvastatin (dotted line): 5, 10, 15, 20, and 25 μg/mL, and rosuvastatin (solid line): 10, 15, 20, 25, and 30 μg/mL.

Ratio spectra-CWT method (RS-CWT). According to the research design, we studied the standard spectra of RVS at different concentrations for identifying the optimum divisor to treat AT ratio. Then the RVS standard spectrum for the concentration of 15 μg/mL was found to be appropriate for achieving RS of AT and its binary mixtures. Similarly, the standard spectra of AT in diverse concentrations have been studied to assay the optimum divisor of RS in determining RVS, and the standard spectrum of 10 μg/mL was selected. Next, multiple CWT families at distinctive scaling parameters (a) have been investigated and utilized by RS. Moreover, the findings reflected the suitability of RS-fk8-CWT (a = 32) and RS-morl-CWT (a = 16) to analyze AT and RVS in the presence of each other. In addition, CWT coefficients of RS against wavelengths have been plotted to construct RS-fk8-CWT and RS-morl-CWT spectra. The linear calibration equations

have been calculated with the measurement of the signal amplitudes in the zero-crossing points: 241 and 285 nm in RS-morl-CWT and RS-fk8-CWT in AT, and also 292 and 295 nm in RS-morl-CWT and RS-fk8-CWT in RVS respectively. Linear regression analysis and its statistical outputs to utilize RS-CWTs are summarized in Table 1. According to the table, the correlation coefficients (r) were between 0.9992 and 1.000, and the standard errors of calibration dependences (slope, intercept, and correlation) were also lower than 0.06. The slopes of calibration curves lay within a range of 0.0465 to 0.3445. These values were better and comparable to those of other RS-CWT and CWT techniques. Furthermore, LODs and LOQs were appropriate values.

Derivative spectrophotometry (DS). Processing the original UV spectra (200–350 nm) has been chosen to obtain the DS_1 spectra of AT and RVS in the concentration ranges of 5–25 and 10–30 μg/mL, respectively, which are illustrated in Fig. 3. According to the figure, calibration graphs for both drugs have been plotted via measuring the derivative intensity at 295 and 327 nm (the zero-crossing points for determining AT and RVS). Table 1 reports the calibration graphs and the statistical results.

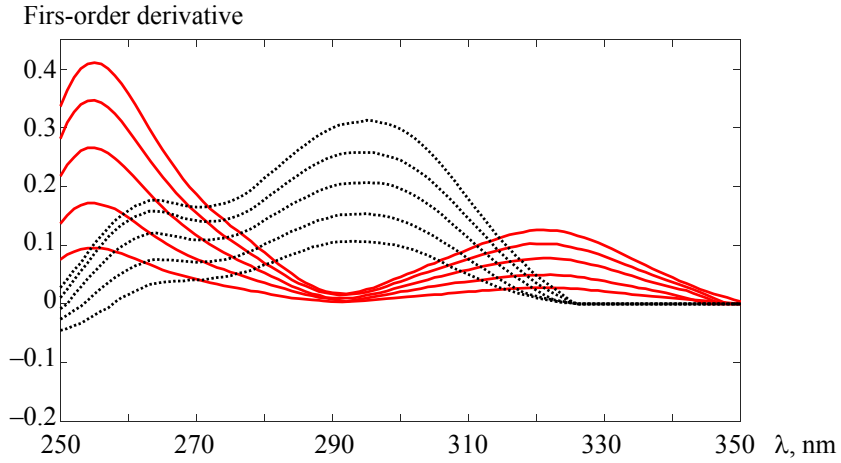

Fig. 3. First-order derivative spectrometry spectra of atorvastatin (dotted line): 5, 10, 15, 20, and 25 μg/mL, and rosuvastatin (solid line): 10, 15, 20, 25, and 30 μg/mL.

Method validation. For method validation, a number of analytical parameters such as accuracy, linearity, LOD, and LOQ must be calculated. It is notable that the above parameters have been utilized for demonstrating the function and the utility of the recommended signal processing procedures. Then, the SDs of slopes and intercepts of the regression lines were calculated to obtain LOD and LOQ values. Table 1 shows that the linear concentration range 5–25 μg/mL for AT and 10–30 μg/mL for RVS are appropriate for analyzing the drugs with good linearity ($r^2 > 0.99$) and acceptable LODs and LOQs. Moreover, the analytical parameters of the proposed methods were compared with DS_1 for the simultaneous determination of AT and RVS. The mean of percent recoveries as well as their relative standard deviations (RSDs) have been computed (Table 2). Satisfactory mean of data recovery, which was achieved to utilize two signal processing methods (CWTs and DS_1), indicated the reasonable accuracy of the method with any remarkable interferences of available excipients in the combined formulations of what was being studied. In addition, satisfactory precisions of LODs and LOQs have also been observed in the newly designed spectrophotometric techniques. However, it was observed that CWT can achieve better results than the DS_1 method. Generally, this can be justified due to the signal amplification in CWT methods.

Finally, we conducted some statistical type of analysis of CWT outputs, and the results were detected in HPLC as a reference method for the simultaneous determination of AT and RVS in their mixed formulation. The results obtained were compared in terms of mean and standard deviation using t-test and F-test, respectively. It was seen (Table 3) that any significant differences have not been observed between the results at 95% of confidence level.

TABLE 1. Linear Least Square Analysis and Statistical Results

Parameter	Meyer-CWT		Gaus8-CWT		RS-morl-CWT		RS-fk8-CWT		DS_1	
	AT	RVS	AT	RVS	AT	RVS	AT	RVS	AT	RVS
λ, nm	338	304	318	278	241	292	285	295	295	327
Range, μg/mL	5–25	10–30	5–25	10–30	5–25	10–30	5–25	10–30	5–25	10–30
m	0.0592	0.0795	0.0804	0.0766	0.0465	0.212	0.1186	0.3445	0.0104	0.0049
n	0.3173	–0.4172	0.3464	–0.3399	0.9773	–0.832	0.6976	–1.744	0.0522	–0.0226
r	0.9992	0.9990	0.9994	0.9995	0.9984	1.000	0.9998	0.9999	0.9997	0.9992
SE (*m*)	0.0004	0.0004	0.0077	0.0008	0.0005	0.0007	0.0018	0.0003	0.0002	0.0002
SE (*n*)	0.0074	0.0186	0.0573	0.0084	0.0089	0.0155	0.0135	0.0155	0.0046	0.0019
SE (*r*)	0.0071	0.0139	0.0544	0.0063	0.0085	0.0115	0.0130	0.0115	0.0044	0.0014
LOD, μg/mL	0.49	0.54	0.84	0.51	0.56	0.87	0.39	0.80	0.65	1.11
LOQ, μg/mL	1.67	1.82	2.82	1.70	1.87	2.88	1.31	2.67	1.98	3.39

N o t e: AT: Atorvastatin; RVS: Rosuvastatin; CWT: Continuous wavelet transform; RS: Ratio spectra; Gaussian: gaus; Fejer korovkin (fk); Morlet: morl; *m*: Slope of the straight-line; *n*: *y*-intercept of the straight line; *r*: Correlation coefficient; SE: Standard error; LOD: Limit of detection; LOQ: Limit of quantitation; DS_1: First-order derivative spectrometry.

TABLE 2. The Percent Recovery Data of Atorvastatin and Rosuvastatin in the Synthetic Mixtures by Applying the Signal Processing Developed Methods

Mixture, μg/mL		Meyer-CWT		Gaus8-CWT		RS-fk8-CWT		RS-morl-CWT		DS_1	
AT	RVS	AT	RVS	AT	RVS	AT	RVS	AT	RVS	AT	RVS
6	16	97.3	99.6	102.1	96.4	103	99.6	99.3	101	94.2	95.6
7	20	104.3	106.4	98.3	99.9	105.6	98.8	104.5	96.5	97.8	96.9
10	13	101.5	101.6	102.3	96.8	103	101	101.2	97.8	101.2	99.1
14	12	103	99.3	98.8	101	97.6	102	97.3	105	96.4	103
10	10	103.3	105	103.6	95.9	104.3	102	102	100.2	95.2	93.6
5	10	100.8	102.6	103.3	97.3	101.6	96.5	103.6	106	98.5	102.7
15	18	96.2	95.9	97.9	102	97.3	105	98.5	105.3	102.1	96.5
Mean		100.9	101.4	100.9	98.4	101.7	100.7	100.9	101.6	97.9	98.2
SD		2.85	3.32	2.28	2.26	2.96	2.51	2.47	3.53	2.72	3.30
RSD%		2.82	3.27	2.25	2.29	2.91	2.49	2.44	3.47	2.77	3.36

TABLE 3. Statistical Comparison of the Results Obtained by the Proposed Methods (CWTs) and HPLC for the Analysis of AT and RVS in their Mixed Formulation

Method	AT	RSV
HPLC[a]	98.3 ± 0.7	95.0 ± 1.3
CWTs[b]	99.5 ± 0.4	97.4 ± 1.6
t_{exp}	2.57	2.66
F_{exp}	3.06	1.50

[a] Prepared of Lipitor: AT 20 mg and Crestor: RVS 20 mg.
[b] Mean percent recovery results ± standard deviation, obtained with the HPLC and proposed CWT techniques. $t_{table} = 2.78$ and $F_{table} = 19$ for 95% confidence level.

Conclusions. The quantitative analyses of two-component compounds with overlapping peaks require a pre-separation technique before determining. The need for using solvents is time-consuming. A simple solution was proposed by using spectral signal processing methods to overcome overlapped spectra problems without a need for primary separation. The UV spectrophotometric method was developed and evaluated to

resolve AT and RVS spectra in the respective binary mixtures free from the requirement of any chemical separation stage and based on DS_1, CWT (Meyer and gaus8), and RS-CWT (morl and fk8). According to the obtained results, the recommended signal processing procedures offer successfully experimental outputs. These proposed methods are also simple and rapid and can be used for the simultaneous detection of AT and RVS in pure and combined pharmaceutical forms. Notably, CWTs is more feasible for discovering the zero-crossing points in comparison with DS_1 using greater numbers of the signal amplification and the wavelet families. CWT methods also presented some advantages, including precision, accuracy, LOD, and LOQ to manage quality and to routinely analyze the studied drugs in comparison with DS_1 procedure. The results of the developed method were statistically comparable to HPLC in terms of accuracy and precision.

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