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Simultaneous Spectrophotometric Determination of Metformin Hydrochloride and Glibenclamide in Binary Mixtures Using Combined Discrete and Continuous Wavelet Transforms

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In this work, a combined discrete and continuous wavelet transform analysis was developed for simultaneous spectrophotometric determinations of metformin hydrochloride and glibenclamide, two antidiabetic drugs, in binary mixtures without any chemical pretreatment. Absorption spectra were subjected to the 4-level db4 discrete wavelet transform (DWT) for signal de-noising. Selected continuous wavelet transform (CWT) families (rbio3.1 with scaling factor, a = 80, and gaus2, a = 60) were applied on these de-noised signals. Finally, a zero-crossing technique was used for the construction of calibration curves for both drugs. The proposed method was validated by analyzing synthetic mixtures of the investigated drugs with various concentrations. The amount of metformin hydrochloride and glibenclamide were determined by using CWT amplitudes in zero-crossing points. The mean recovery values of metformin hydrochloride and glibenclamide were found between 98.6 – 102.0 and 97.9 – 102.4% for rbio3 and 98.3 – 101.2 and 97.1 – 101.4% for gaus2 families, respectively. The obtained results showed that the developed method is a simple, rapid and precise procedure for the simultaneous determination of metformin hydrochloride and glibenclamide in binary mixtures.

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Introduction

Metformin hydrochloride (1-(diaminomethylidene)-3,3-dimethyl-guanidine hydrochloride), is one of the most important drugs for the treatment of type II diabetes mellitus. Glibenclamide (5-chloro-*N*-[2-[4-(cyclohexylcarbamoyl-sulfamoyl)phenyl]-ethyl]-2-methoxy-benzamide), a sulfonylurea antidiabetic drug, is also widely used to treat type II diabetes mellitus.[1] It has been shown that the combination of metformin with sulfonylureas (*e.g.* glibenclamide, gliclazide, *etc.*) is often superior to therapy with a single antidiabetic agent.[2-4] This finding is important because single-drug therapy often fails to maintain normoglycemia, particularly as diabetes progresses. As diabetes progresses and treatment with maximal doses of sulfonylurea fails, the addition of metformin significantly improves glycemic control.[5-7] There are various commercial pharmaceutical products based on metformin and glibenclamide on the market.

The simultaneous determinations of metformin and glibenclamide in their mixtures with or without other compounds has been investigated by means of reversed-phase high-performance liquid chromatography (RP-HPLC),[8] liquid chromatography/(atmospheric pressure chemical ionization) mass spectrometry (LC/(APCI)MS),[9] liquid chromatography tandem mass spectrometry (LC-MS/MS),[10] a HPLC method based on UV detection and an ion-pair solid-phase extraction technique[11] and nonaqueous solid-phase extraction capillary electrophoresis technique.[12] These complex HPLC methods are rather expensive, time consuming, and regarding to the complexity of experimental conditions, found not to be suitable. On the other hand, due to very strong overlapping between the absorption spectra of the two investigated drugs in this study, simple spectrophotometry is not suitable for simultaneous determination. Wavelet transform techniques can effectively solve this drawback.[13,14]

Wavelet transform is a strong tool for signal de-noising[15] and baseline removal,[16] signal compression and processing, and multicomponent analysis; it has been established as a powerful technique in analytical chemistry.[17-20] By means of wavelet transform, an original signal can be decomposed into localized contributions characterized by a scale parameter. Each contribution represents a portion of the signal with a different frequency. Furthermore, the wavelet transform is a linear operation, which is important for quantitative analysis.

Wavelet transform methods have been successfully used for the resolution of overlapped spectra for the quantitative determination of multicomponent mixtures by means of chromatographic methods,[21-24] spectrophotometry,[25-34] electrochemical methods,[35-41] *etc.* Also, discrete wavelet transform is a promising technique for de-noising analytical signals.[25,42]

In the present work, we developed a method based on the combination of discrete wavelet de-noising and continuous wavelet transform using spectrophotometric data for the simultaneous determination of metformin hydrochloride and glibenclamide in binary mixtures. The proposed method consists of four individual steps: recording UV-spectra of standard and test solutions; de-noising all spectral signals using one-dimensional discrete wavelet transform; applying continuous wavelet transform on the de-noised signals and

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obtaining new signals with zero-crossing points; and finally, using a zero-crossing technique for producing calibration graphs for both investigated compounds. The method was applied to a series of mixtures of metformin hydrochloride and glibenclamide.

Theory

Wavelet transform analysis

Wavelet transform aims at transforming a signal from the original domain to another one in which operations on the signal can be carried out more easily.[43]

De-noising by discrete wavelet analysis (DWT)

The original signal f is decomposed in many sub-signals, the first of which is called the trend signal, and the others are called fluctuations. Using a k-level multiresolutional analysis, if the number N of signal values is divisible k times by 2, the signal is decomposed as

$$f = A^{k} + D^{k} + ... + D^{l}, \quad (1)$$

where A^{k} represents the obtained trend signal and $D^{k} + ... + D^{l}$ represents the fluctuations.[44-46] Generally, the noisy signal f can be expressed as

$$f = s + n, \quad (2)$$

where f is the noisy signal, s represents the original signal and n denotes the noise signal.

Continuous wavelet transform (CWT)

The original wavelet function is known as the mother wavelet, and is used to generate all basis functions.[47,48] A wavelet is expressed as a series of functions, $\Psi_{a,b}(\lambda)$ having the following forms:

$$\Psi_{a,b}(\lambda) = \frac{1}{\sqrt{|a|}}\Psi\left(\frac{\lambda - b}{a}\right) \qquad a \neq 0 \quad a,b \in R. \quad (3)$$

Here a represents a scale parameter, which is a positive variable, used to control the scaling, and b represents the translation parameter used for shifting. In other words, there is a set of functions, $\Psi_{a,b}(\lambda)$ obtained from a mother wavelet by scaling (dilatation) and shifting (translation).

Wavelet transforms contain the computations of coefficients obtained from the inner products of the signal and a family of wavelets. The CWT of signal $f(\lambda)$ is defined as

$$\mathrm{CWT}\{f(\lambda);a,b\} = \int_{-\infty}^{+\infty} f(\lambda)\psi^{*}_{a,b}(\lambda)\mathrm{d}\lambda = \langle f(\lambda)\psi_{a,b}\rangle, \quad (4)$$

where the superscript $*$ represents the complex conjugate, $\psi^{*}_{a,b}$ is a translated and scaled complex-conjugated mother wavelet, and $\langle f(\lambda)\psi_{a,b}\rangle$ denotes the inner product of the function $f(\lambda)$ on the wavelet function $\psi_{a,b}(\lambda)$.

Experimental

Reagents and chemicals

Pharmaceutical-grade metformin hydrochloride and glibenclamide were kindly donated by Chemidarou Pharmaceutical Co. (Tehran, Iran). Methanol (analytical grade) was obtained from Merck (Germany).

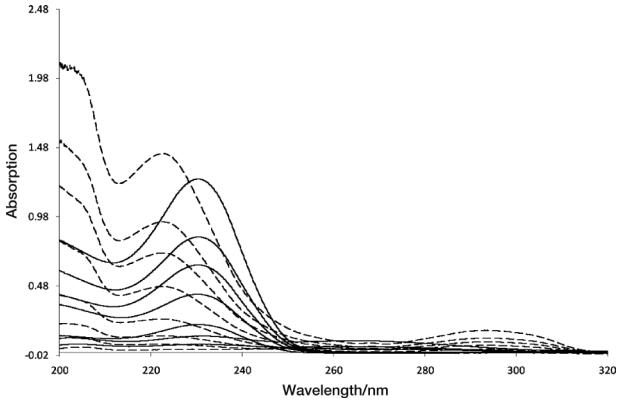

Fig. 1 Absorption spectra of metformin hydrochloride 0.5, 1.0, 2.5, 5, 7.5, 10, 15, 20 mg L^{-1} (-) and 0.5, 1, 2, 5, 10, 15, 20, 30 mg L^{-1} glibenclamide (- - -) in methanol.

Apparatus

A Bio-TEK Kon 922 double-beam spectrophotometer connected to a computer loaded with UV-Vis Analyst software was used for recording the spectra. Ultraviolet spectra of standard and sample solutions were recorded in 1-cm quartz cells over the range of 200 – 400 nm.

The calculations and wavelet transforms were performed by using Excel 2007 and MATLAB 7.8 software.

Standard solutions and binary mixtures

A series of standard solutions containing 0.5 – 20 mg L^{-1} of metformin hydrochloride and 0.5 – 30 mgL^{-1} of glibenclamide were prepared from stock solutions of 100 mg L^{-1} by diluting accurate volumes of the stock solution in methanol. A set of binary mixture solutions in the presented range for both compounds was prepared by mixing the proper volume of stock solutions and diluting with methanol.

Results and Discussion

Spectrophotometric analysis

Absorption spectra of standard solutions and binary mixtures of investigated drugs were recorded in the range of 200 – 400 nm. Figure 1 shows strong overlapping in UV spectra of metformin hydrochloride and glibenclamide. Hence, simultaneous determinations of investigated compounds by the classical spectrophotometric method were not possible to perform. Wavelet transform analysis was applied for the quantitative resolution of binary mixtures of the investigated compounds.

De-noising by discrete wavelet transform

In this work, a soft thresholding method was used for signal de-noising. To eliminate the background noise of the spectrophotometric signals, they were subjected to several wavelet transform families; among them, 4-level discrete daubechies 4 (db4) wavelet transform analysis showed the best results. The de-noised signals is shown in Fig. 2. The threshold values for metformin hydrochloride, glibenclamide and mixtures were 0.002, 0.003, and 0.001, respectively.

Continuous wavelet transform

A continuous wavelet transform of the de-noised signal produces a transformed signal consisting several zero-crossing points. The de-noised spectral signals were processed by

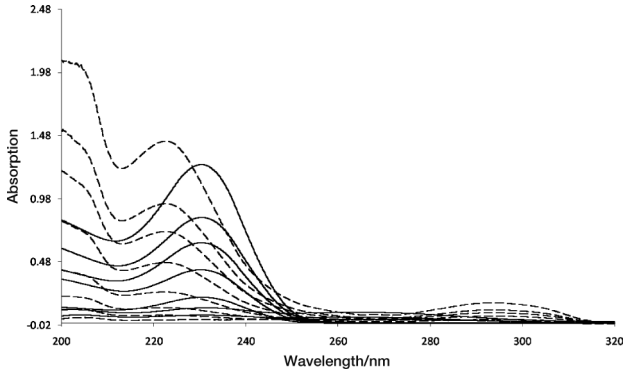

Fig. 2 Discrete wavelet de-noised spectra of metformin hydrochloride 0.5, 1.0, 2.5, 5, 7.5,10, 15, 20 mg L^{-1} (-) and 0.5, 1, 2, 5, 10, 15, 20, 30 mg L^{-1} glibenclamide (- - -) in methanol.

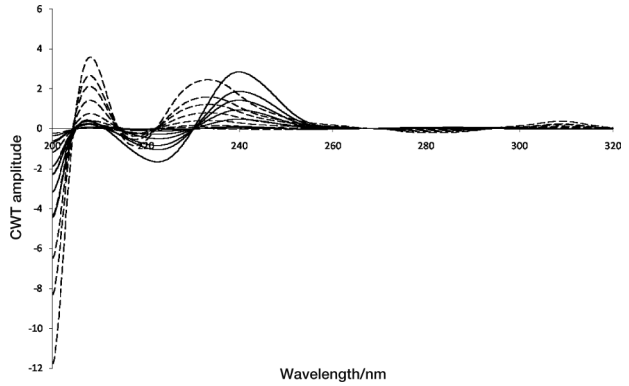

Fig. 3 Continuous wavelet transformed rbio3.1 (a = 60) spectra of 0.5, 1, 2.5, 5, 7.5, 10, 20 mg L^{-1} metformin hydrochloride (-) and 0.5, 1, 2, 5, 10, 15, 20, 30 mg L^{-1} glibenclamide (- - -) in methanol.

various continuous wavelet families with different scale parameters (a) to find the optimal conditions, where the zero-crossing point of the wavelet transformed signal corresponding to one component corresponds to a peak in the signal of another compound. Reversed biorthogonal 3.1 (rbio3.1) with the scale parameter a = 60 and Gausian 2 (gaus2) with a = 80 were selected for further analysis as optimal wavelet families. CWT graphs for metformin and glibenclamide obtained by rbio3.1 and gaus2 are shown in Figs. 3 and 4, respectively.

Calibration graphs for each compound were obtained by plotting the CWT amplitude of the transformed signal of the compound *versus* the concentration at the zero-cross point of the other compound. Linear calibration graphs for the determination of metformin hydrochloride (0.5 – 20 mg L^{-1}) and glibenclamide (0.5 – 30 mg L^{-1}) with rbio3.1 were obtained by measuring the CWT amplitudes at 222.1 and 230.1 nm, corresponding to the zero-crossing points, respectively (Figs. 5a and 5b). Also, calibration graphs were plotted by gaus2, by measuring the CWT signal amplitude at 234.2 nm for metformin hydrochloride and 241.2 nm for glibenclamide (Figs. 6a and 6b).

Table 1 summarizes the linear-regression analysis and its statistical results for the quantification of metformin and glibenclamide, using selected CWT families. The correlation coefficients of calibration graphs were found to be higher than 0.999 for both compounds and two proposed wavelet families.

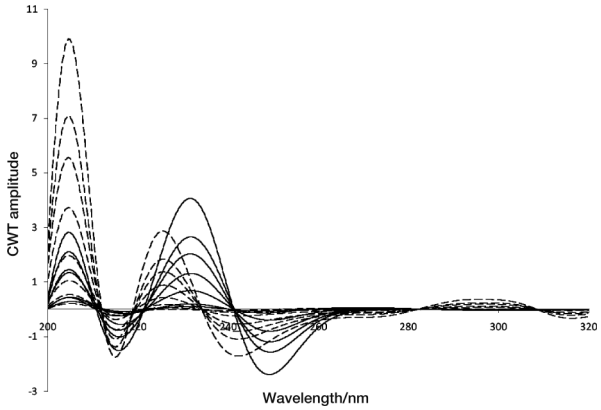

Fig. 4 Continuous wavelet transformed gaus2 (a = 80) spectra of 0.5, 1, 2.5, 5, 7.5, 10, 20 mg L^{-1} metformin hydrochloride (-) and 0.5, 1, 2, 5, 10, 15, 20, 30 mg L^{-1} glibenclamide (- - -) in methanol.

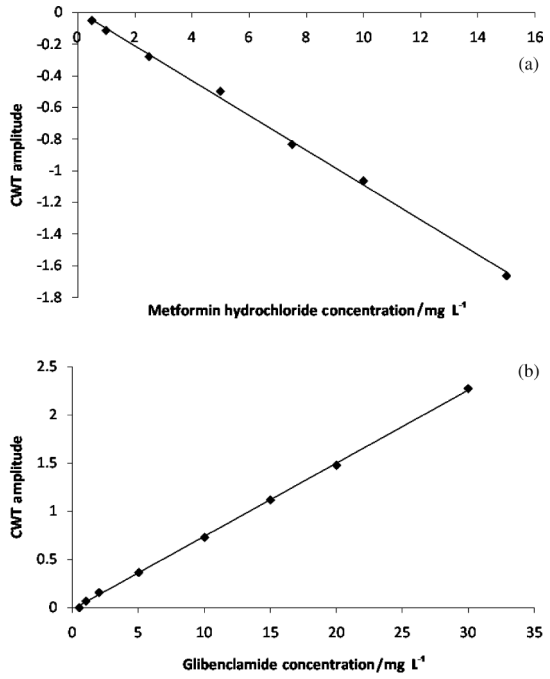

Fig. 5 rbio3.1 linear calibration graphs for determination of (a) metformin hydrochloride at 222.1 nm, and (b) glibenclamide at 230.1 nm.

Multicomponent resolution of binary mixtures

The proposed method for the simultaneous determinations of metformin and glibenclamide was tested by analyzing a set of synthetic mixtures of the compounds within the linear range of the calibration graphs. Table 2 gives the results and a statistical analysis of the method validation process.

As shown in Table 2, the accuracy and precision of the proposed method were analyzed by evaluating the mean recoveries. The obtained results show good accuracy and reproducibility for both wavelet families. It was observed that the results of the quantification of mixture solutions had good agreement with each other. The mean recovery values of metformin hydrochloride and glibenclamide were found between 98.6 – 102.0 and 97.9 – 102.4% for rbio3 and 98.3 – 101.2 and 97.1 – 101.4% for gaus2 families, respectively.

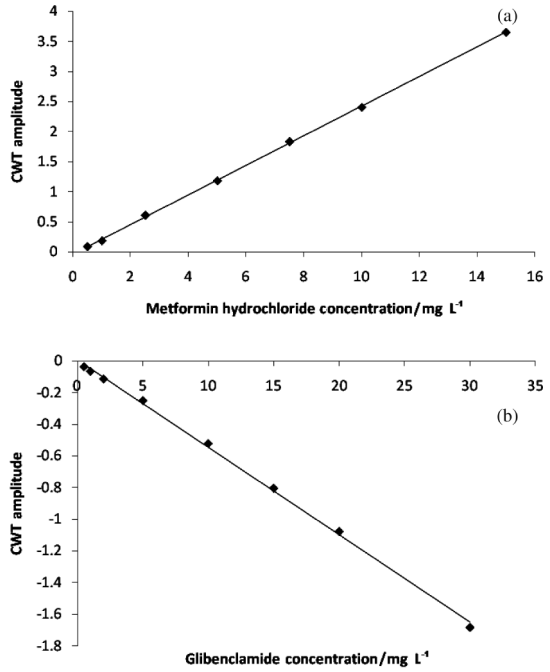

Fig. 6 gaus2 linear calibration graphs for determination of (a) metformin hydrochloride at 234.2 nm, and (b) glibenclamide at 241.2 nm.

Table 1 Linear regression analysis and its statistical data

Parameter	rbio3.1		gaus2	
	Metformin	Gliben-clamide	Metformin	Gliben-clamide
Wavelength/nm	222.1	230.1	234.2	241.2
Range/mg L^{-1}	0.5 – 20	0.5 – 30	0.5 – 20	0.5 – 30
Slope	–0.11	0.075	0.246	–0.055
Intercept	0.01	–0.015	–0.037	0.008
Correlation coefficient (r)	0.9990	0.9995	0.9995	0.9990
Limit of detection (LOD)/mg L^{-1}	0.11	0.15	0.09	0.13
Limit of quantification (LOQ)/mg L^{-1}	0.33	0.45	0.27	0.39

The accuracy and the precision of the wavelet analysis were confirmed by applying the standard addition technique by the addition of known amounts of the investigated compounds to a known binary mixture of metformin hydrochloride and glibenclamide. The obtained results of both wavelet families were compared with each other using the one-way ANOVA test (Table 3). The calculated F-values for all mixtures were less than the tabulated F-values, which confirms that no significant errors were observed in the determination of drugs by the proposed methods at the 95% confidence level.

Conclusions

A combination of de-noising spectral signal by discrete wavelet transform and continuous wavelet transform of de-noised spectrophotometric signals, followed by a zero-crossing technique, was applied for the simultaneous determination of metformin hydrochloride and glibenclamide in binary mixtures. The results showed that using these techniques can determine the concentrations of both compounds precisely and accurately, despite the strong overlapping spectra. The optimal processing conditions were obtained using db4 DWT, followed by rbio3.1 (a = 60) or gaus2 (a = 80) CWT analysis.

The presented results show that this method is a simple, rapid and reliable for the simultaneous determination of metformin hydrochloride and glibenclamide, for the quality control and routine analysis of binary mixtures and commercial products.

Table 2 Recoveries of metformin and glibenclamide in binary mixtures

Concentration/ mg L^{-1}		Recovery, %			
		rbio3.1 (a = 60)		gaus2 (a = 80)	
Metformin	Gliben-clamide	Metformin	Gliben-clamide	Metformin	Gliben-clamide
1	10	102.0	98.8	99.1	97.1
2	10	101.4	97.9	100.2	98.5
5	10	100.7	98.6	98.8	99.3
10	10	101.1	99.0	101.0	98.9
20	10	99.8	98.1	98.3	99.4
15	5	98.7	100.2	98.6	100.6
10	1	100.2	102.4	100.5	100.9
10	2	100.6	101.3	101.2	101.4
10	5	99.7	99.6	100.9	98.7
5	15	98.6	98.7	98.7	99.8
Mean		100.3	99.5	99.7	99.5
RSD[a]		1.105	1.455	1.138	1.283

a. Relative standard deviation.

Table 3 The ANOVA analysis for two CWT families

Concentration/mg L^{-1}		F_{crit}	F	
Metformin	Glibenclamide		Metformin	Glibenclamide
1	10	7.708	0.1328	1.2282
2	10		0.0106	0.0771
5	10		0.0205	0.4461
10	10		0.0154	0.1555
20	10		0.0106	0.0601
15	5		0.5086	0.0144
10	1		3.8272	0.0568
10	2		0.5793	0.0178
10	5		3.4554	0.1077
5	15		0.5858	0.0518

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