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Spectrophotometric Determination of Ramipril and Hydrochlorothiazide in a Mixture

ERDAL DINÇ[1,*] and DUMITRU BALEANU[2,3]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey
[2]Department of Mathematics and Computer Sciences, Faculty and Art and Sciences, Çankaya University, 06530 Ankara, Turkey
[3]National Institute for Laser, Plasma and Radiation Physics, Institute of Space Science, Magurele-Bucharest, P.O.Box, MG-23, R 76911, Romania

*Corresponding author: Fax: +90 312 2131081; Tel: +90 312 2154886; E-mail: dinc@pharmacy.ankara.edu.tr

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Double wavelet transforms based on the use of continuous wavelets transform (CWT) and discrete wavelets transform (DWT) were proposed for the simultaneous quantification of hydrochlorothiazide and ramipril in tablets. DMEY-CWT was applied to the simultaneous quantitative resolution of the overlapping spectra of the UV spectra of the related compounds and their two-component mixtures. After that DMEY-CWT spectra were processed by BIOR3.5-DWT due to the noises and fluctuations coming from instrumentation and experimental media, respectively, to obtain the smoothing and denosing double transformed signals. Calibration graphs for ramipril and hydrochlorothiazide were obtained by measuring the double transformed wavelet amplitudes at 254.1 and 246.4 nm in the spectral region 230.0 and 281.1 nm, respectively. The validation of the continuous wavelets transform signal processing method was performed on the recovery study. The proposed method was successfully applied to the commercial tablets containing two compounds and good accuracy and precision were reported.

Key Words: Continuous and discrete wavelet transforms, Spectrophotometric determination, Ramipril, Hydrochlorothiazide.

INTRODUCTION

Wavelet transform (WT) have become a powerful tool used in various fields of science and engineering and it consists of the discrete and the continuous transforms[1]. During the previous years the continuous wavelets transform (CWT) approach started to be recognized as a powerful signal processing method for data reduction, de-noising, baseline correction as well as for the resolution of overlapping spectra[2,3]. In addition, the combination of the CWT methods with zero-crossing technique and ratio signals started to be applied sucessfully for the simultaneous spectrophotometric resolution of binary and ternary mixtures[4-11].

Ramipril-hydrochlorothiazide combination is used in commercial pharmaceutical formulations to diminish the high blood pressure, namely ramipril and hydrochlorothiazide are used as ACE inhibitor and diuretic agents, respectively. Quantitative analysis of the mixtures consisting of ramipril and hydrochlorothiazide was reported by HPLC[12]. The determination of ramipril in samples with other active compounds was perfromed by spectrophotometric and atomic absorption[13].

In this study, a new approach based on the use of continuous wavelets transform (CWT) combined with discrete wavelets transform (DWT) was devloped and applied to the simultaneous spectrophotometric determination of ramipril and hydrochlorothiazide in the commercial pharmaceutical tablets witout any separation step. As a comparison method the fourth derivative spectrophotimetry (DS4) was used for the quantitative evaluation of ramipril and hydrochlorothiazide in their mixture. After the validation procedure the proposed wavelet and derivative methods were sucessfully applied to the simultaneous analysis of ramipril-hydrochlorothiazide tablets.

EXPERIMENTAL

The absorption spectra of the compounds and their corresponding samples were obtained by making use of a Shimadzu UV-160 double beam UV-vis spectrophotometer having a fixed slit width (2 nm), connected with a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer. For CWT and DWT approaches, the Microsoft EXCEL and Wavelet Toolbox in Matlab 7.0 software were used for data treatments, regressions and statistical analysis.

Commercial pharmaceutical formulation: A commercial pharmaceutical sample (Blokace® Plus Tablet, Actavis Group hf.-Islanda (produced by Fako Ilaçlari A.S.) Batch No: F13623) containing 2.5 mg ramipril and 12.5 mg hydrochlorothiazide per tablet was analyzed.

Standard solutions: Stock standard solutions of ramipril and hydrochlorothiazide were separately prepared by dissolving 25 mg of each drug in 100 mL methanol. A standard series of each compound in the concentration range 2.0-22.0 µg/mL for ramipril and 2.0-32.0 µg/mL for hydrochlorothiazide in the above solvent was prepared from the stock standard solutions. An independent validation set of 12 synthetic mixture solutions of ramipril and hydrochlorothiazide in the above working concentration ranges was prepared.

Analysis of commercial samples: Twenty tablets were accurately weighed by using an electronic balance. Tablet content was powdered in a mortar. An amount containing ramipril and hydrochlorothiazide corresponding to one tablet content was dissolved in methanol and made up in 100 mL calibrated flask. This flask containing both compounds was mechanically shaken for 0.5 h and filtrated into a 100 mL volumetric flask through a 0.45 µm membrane filter. The resulting sample solution was diluted to the working concentration range. This procedure was repeated eight replicates. The UV spectra of the tablet samples were recorded processed by DMEY-CWT combined with BIOR3.5 DWT and DS4 methods for the qunatitative evaluation of ramipril and hydrochlorothiazide in tablets.

RESULTS AND DISCUSSION

In the qualitative and quantitative resolution of the complex samples, one of the promssing candidates for the spectral analysis is the wavelet method. Keeping in mind we have foccused on finding the optimal applications of the CWT approach to the spectral wavelet analysis of two-component mixtures consisting of ramipril and hydrochlorothiazide. Actually, the quantitative resolution of the ramipril and hydrochlorothiazide mixtures is not possible by using the classical absorbance measurement in presence of the overlapping spectra. In this case the CWT approach have a good potential power than classical signal analysis methods for the quantitative spectral resolution of strongly overlapping bands of the active compounds in samples.

In this present study several wavelet famillies were tested for finding the optimal signal analysis, namely to obtain a good determination results of ramipril and hydrochlorothiazide.

After finishing the testing procedure, the DMEY-CWT approach was found to be suitable for the analysis of the subject matter compounds. After we have applied the transformation of DMEY-CWT on the subjected compounds. Some signal deformation was observed mainly due to the presence of noise. To resolve this problem several wavelet families have tested.

As a result BIOR3.5-DWT tool was found the best family for the elimination and smoothing of DMEY-CWT spectra and then we have obtained DMEY-CWT- BIOR3.5-DWT spectra for determination of the related compounds.

Continuous and discrete wavelets transform: The UV spectra of compounds between 2.0-22.0 µg/mL of ramipril and 2.0-32.0 µg/mL hydrochlorothiazide in methanol were recorded in the spectral region of 200.0-305.0 nm. Fig. 1 indicates the overlapping UV spectra of ramipril and hydrochlorothiazide in the above mentioned concentration ranges for both compounds. Analogous spectral registrations were applied to the sample solutions. Before applying the wavelet

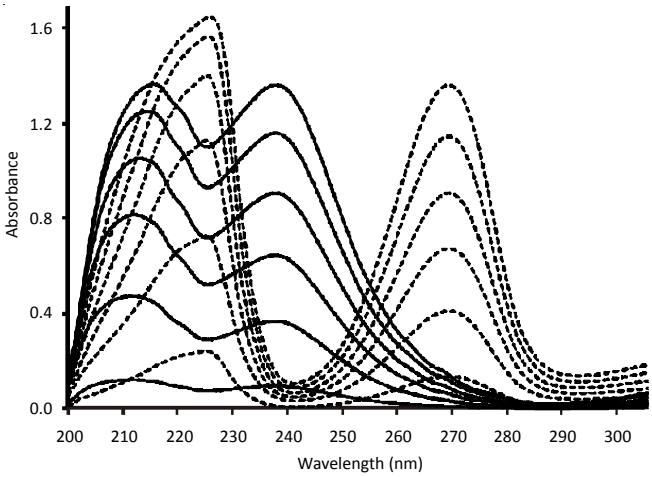
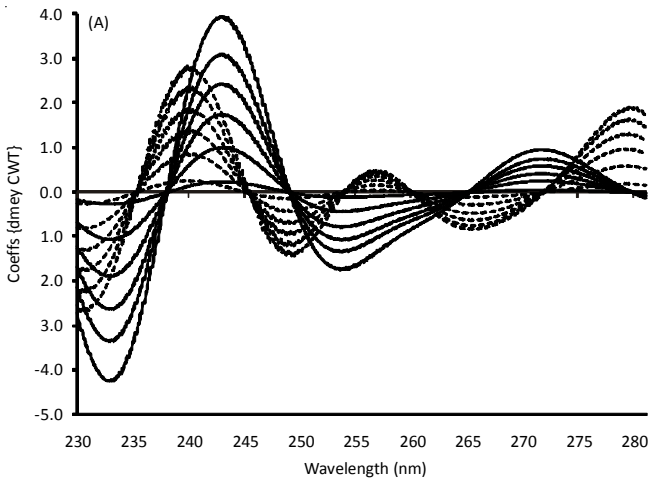


Fig. 1. UV-spectra of ramipril and hydrochlorothiazide in the linear concentration range of 2.0-22.0 and 2.0-32.0 µg/mL in methanol

transform to the UV spectra of compounds and their sample solutions various continuous wavelet families for different scale parameters (a) were tested and then the DMEY-CWT (a = 150) was found to be optimal transformation of the UV spectra to provide a good resolution and determination of ramipril and hydrochlorothiazide in samples. Fig. 2A shows the DMEY-CWT spectra of the ramipril and hydrochlorothiazide compounds in the concentration range of 2.0-22.0 µg/mL for ramipril and 2.0-32.0 µg/mL for hydrochlorothiazide. After that BIOR-DWT (which is an optimal signal analysis tool) at the level equals to 4 was applied to the DMEY-CWT spectra to smooth the spectral fluctuations and to eliminate the spectral noises from instrumentation registration.

The DMEY-CWT-BIOR3.5-DWT spectra of compounds between 2.0-22.0 µg/mL of ramipril and 2.0-32.0 µg/mL of hydrochlorothiazide were presented in Fig. 2B. In application of the double transform based on DMEY-CWT and BIOR3.5-CWT the calibration graphs were obtained by measuring the DMEY-CWT-BIOR3.5-DWT amplitudes at 254.1 nm for ramipril and 246.4 nm for hydrochlorothiazide. At the above selected points the least squares regression analysis and its statistical results were summerized in Table-1. ramipril and hydrochlorothiazide in their samples were determined by using the above calculated calibration graphs.



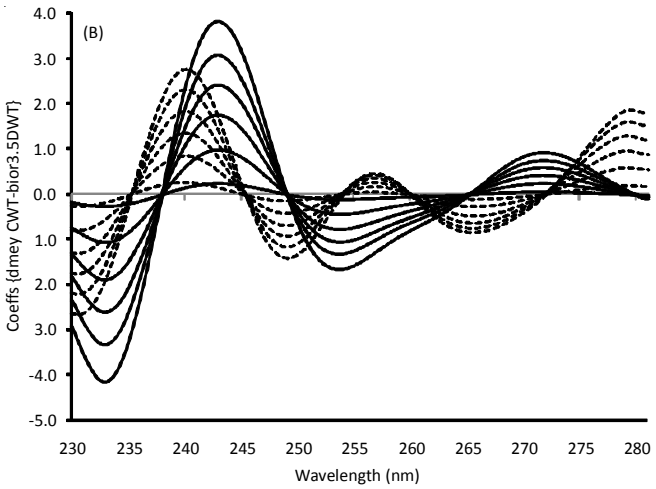


Fig. 2. (A) DMEY-CWT spectra and (B) DMEY-CWT-BIOR3.5-DWT spectra of ramipril and hydrochlorothiazide in the concentration ranges of 2.0-22.0 and 2.0-32.0 µg/mL, respectively

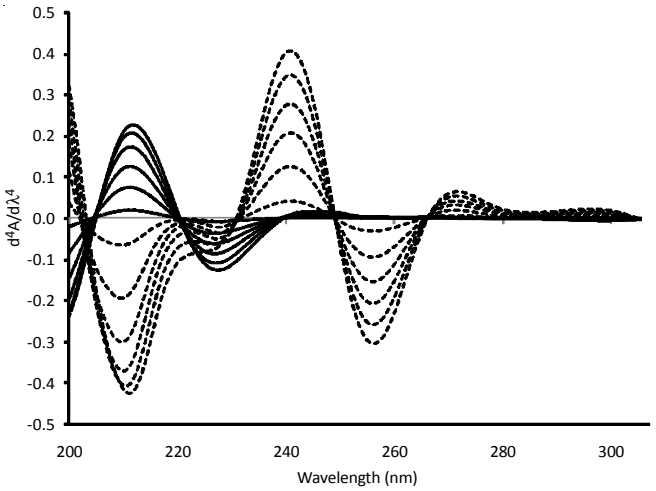


Fig. 3. Fourth derivative spectra of ramipril and hydrochlorothiazide in the concentration levels between of 2.0-22.0 µg/mL of ramipril and 2.0-32.0 µg/mL of hydrochlorothiazide, respectively (Δλ = 12 nm scaling factor equals to 4 and the smoothing interval equals 5 were used)

TABLE-1
LEST-SQUARES REGRESSION ANALYSIS RESULTS FOR THE DETRMINATION OF RAM AND HCT BY THE PROPOSED WAVELET AND DERIVATIVE METHODS

	DMEY CWT-BIOR3.5 DWT		DS4	
	RAM	HCT	RAM	HCT
Range (µg/mL)	2.0-22.0	2.0-32.0	2.0-22.0	2.0-22.0
m	-5.08×10^{-2}	-6.05×10^{-2}	-3.56×10^{-3}	1.89×10^{-2}
n	-2.91×10^{-2}	-3.96×10^{-2}	1.41×10^{-3}	1.04×10^{-2}
r	0.9993	0.9991	0.9997	0.9995
SE(m)	9.42×10^{-4}	1.46×10^{-3}	1.17×10^{-4}	2.99×10^{-4}
SE(n)	1.87×10^{-3}	1.50×10^{-3}	2.03×10^{-4}	6.13×10^{-4}
SE(r)	2.37×10^{-2}	2.44×10^{-2}	2.95×10^{-3}	5.01×10^{-3}
LOD (µg/mL)	0.27	0.18	0.42	0.24
LOQ (µg/mL)	0.90	0.61	1.40	0.80

n = Intercept of the linear regression equation. r = Correlation coefficient of the linear regression equation. SE(m) = Standard error of the slope. SE(n) = Standard error of the intercept. SE(r) = Standard error of the correlation coefficient. LOD = Limit of detection. LOQ = Limit of quantitation.

Fourth derivative spectrophotometry: As shown in Fig. 1, the UV spectra of ramipril and hydrochlorothiazide in methanol are overlapped in the spectral region of 200-305 nm. Derivative spectrophotometry from first to fourth order was applied to the UV spectra of the related compounds in their mixtures. Therefore, DS4 was found to be suitable for the best spectral resolutions of the overlapping spectra of ramipril and hydrochlorothiazide in the above mentioned spectral region between 200-305 nm. DS4 procedure was prformed with the interval of $\Delta\lambda$ =12 nm and the scalling factor equals to 4. Their fourth derivative spectra of ramipril and hydrochlorothiazide, in the range of 2.0-22.-0 µg/mL for ramipril and 2.0-32.0 µg/mL for hydrochlorothiazide are shown in Fig. 3.

In this case the slope and intercept for the linear regression equations with other statistical parameters were calculated by using the relationship between concentration of compounds and the fourth derivative amplitudes at 230.6 nm for ramipril and 239.8 nm for hydrochlorothiazide, least-squares regression and statistical analysis results were given in Table-1. The calculated regression graphs were applied to the quantitative evaluation of ramipril and hydrochlorothiazide samples.

Validation of the wavelet and derivative methods: A method validation based on the linearity, accuracy and precission parameters was performed. A good linearity was reproted for the application of DMEY CWT- BIOR3.5 DWT and DS4 methods in the concentration range of 2.0-22.0 µg/mL of ramipril and 2.0-32.0 µg/mL of hydrochlorothiazide. In the least-squares regression analysis, the numerical values of the correlation coefficients (r) were depicted in Table-1. For the obtained results by the wavelet and derivative methods good accuracy and precision were reported. Thus, the recovery studies were carried out by application of the proposed DMEY CWT-BIOR3.5 DWT and DS4 to the quantitative analysis of 12 mixture solutions by dilution of the stock solutions. The percentage recoveries and their relative standard deviations were shown in Table-2.

The limit of detection (LOD) and the limit of quantitation (LOQ) for each method were illustrated in Table-1. The calculation of these parameters was done by making use of the standard deviation of the intercept and slope values of calibration equations. During the analysis, no interference of the tablet excipients on the determinations was reported. According to the recovery studies and standard addition technique, it was shown that the numerical results obtained by DMEY CWT-BIOR3.5 DWT and were in good agreement with those provided by DS4

Tablet analysis: Assay results obtained by applying the DMEY CWT- BIOR3.5 DWT and DS4 methods to the ramipril -hydrochlorothiazide tablet solutions as in the sample preparation were indicated are indicated in Table-3. Successful results were obtained for the quantitative analysis of commercial tablets containing ramipril and hydrochlorothiazide compounds. A good coincidence was found between the tablet assay results obtained by the proposed waelet and derivative methods. In the tablet assay analysis, the interference of the tablet excipients on the determination of ramipril and hydro-

TABLE-2
RECOVERY RESULTS FOR RAMIPRIL (RAM) AND HYDROCHLOROTHIAZIDE (HCT) IN THE BINARY SYNTHETIC MIXTURES OF RAMIPRIL AND HYDROCHLOROTHIAZIDE BY THE PROPOSED WAVELET AND DERIVATIVE METHODS

No.	Binary mix. (µg/mL)		DMEY CWT-BIOR3.5 DWT				DS4			
			Found (µg/mL)		Recovery (%)		Found (µg/mL)		Recovery (%)	
	RAM	HCT	RAM	HCT	RAM	HCT	RAM	HCT	RAM	HCT
1	2	4	2.00	3.95	100.1	98.8	1.90	4.04	95.2	101.1
2	6	4	5.67	4.09	94.4	102.2	5.93	4.04	98.8	101.1
3	10	4	10.03	3.95	100.3	98.8	10.16	4.02	101.6	100.6
4	14	4	13.81	4.08	98.6	102.1	14.01	3.99	100.0	99.7
5	18	4	17.75	3.75	98.6	93.8	17.66	4.01	98.1	100.4
6	22	4	21.09	3.94	95.9	98.5	20.62	3.89	93.7	97.4
7	20	2	19.55	1.98	97.7	99.0	20.24	2.04	101.2	102
8	20	8	20.52	7.88	102.6	98.5	19.27	7.86	96.3	98.3
9	20	14	19.27	14.07	96.4	100.5	19.31	14.29	96.5	102.0
10	20	20	20.19	20.13	100.9	100.7	19.26	20.83	96.3	104.1
11	20	26	19.82	25.98	99.1	99.9	19.17	26.07	95.9	100.3
12	20	32	20.33	32.38	101.6	101.2	19.00	34.02	95.0	106.3
–	–	–	–	Mean	98.8	99.5	–	–	97.4	101.1
–	–	–	–	SD	2.44	2.24	–	–	2.54	2.38
–	–	–	–	RSD	2.47	2.26	–	–	2.61	2.36

Binary mix. = Binary mixture, SD = Standard deviation, RSD = Relative standard deviation.

TABLE-3
DETERMINATION RESULTS OF RAMIPRIL AND HYDROCHLOROTHIAZIDE IN TABLETS BY THE PROPOSED WAVELET AND DERIVATIVE METHODS

No.	mg/tablet			
	DMEY CWT-BIOR3.5 DWT		DS4	
	RAM	HCT	RAM	HCT
1	2.52	12.88	2.35	12.26
2	2.59	12.29	2.48	12.22
3	2.56	12.10	2.48	12.26
4	2.52	12.40	2.47	12.80
5	2.44	12.35	2.40	12.32
6	2.61	12.51	2.50	11.87
7	2.46	12.36	2.38	12.07
8	2.53	12.40	2.51	12.55
Mean	2.53	12.41	2.45	12.29
SD	0.06	0.22	0.06	0.28
RSD	2.34	1.79	2.50	2.31
SE	0.02	0.08	0.02	0.10
CL	0.04	0.15	0.04	0.20

SD = Standard deviation, SE = standard error, CL = confidential limit (p = 0.05), label claim: 2.50 mg ramipril and 12.5 mg hydrochlorothiazide per tablet.

chlorothiazide was not recorded in application of proposed methods to real tablet samples.

Conclusion

In this manuscript, the applicability and the suitability of the proposed wavelet transforms and fourth derivative spectrophotometry were demonstrated for the first time for the sucessful determination of the new compound consisting of ramipril and hydrochlorothiazide in tablets. The proposed methods can be applied for the routine quality control of the commercial tablets containing ramipril and hydrochlorothiazide.

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