Continuous Wavelet Transform Applied to the Simultaneous Spectrophotometric Determination of Valsartan and Amlodipine in Tablets

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A new signal procesing approach based on continuous wavelet transform (CWT) was proposed for the simultaneous spcetroptometric determination of valsartan and amlodipine in tablets without making use of any chemical separation procedure. After the CWT method was applied to the analyzed spectra, the results were plotted in wavelet domain. Several CWT families were tested and Daubechie10 (db10-CWT) and DMeyer (dmey-CWT) were found suitable for the determination of VAL and AML. The procedure of the selection of CWT family was based on the recovery results of signal analysis. Calibration graphs obtained by the linear regression analysis were performed by measuring the CWT amplitudes at 258.5 and 263.3 nm for VAL by using dmey-CWT and db10-CWT and at 265.7 and 270 nm for AML by using dmey-CWT and db10-CWT respectively. Linear calibration ranges were considered as 2-42 μg/mL for VAL and 2-32 μg/mL for AML respectively. The validation treatments for the proposed CWT methods were performed by analyzing various synthetic mixtures and by using the standard addition technique. Successful determination results were obtained by applying db10-CWT and dmey-CWT methods to the tablet analysis.

Keywords: wavelet transform, valsartan, amlodipine

As it is well known various spectrophotometric methods such as graphical and numerical approaches have been used for the quantitative analysis of samples containing multicomponents. However in some applications or complex mixture analysis the classical graphical and numerical spectroscopic methods do not provide desirable and reliable results. Namely, the derivative spectrophotometry and its modified versions have been used extensively in fast quantitative analysis of mixtures. However, these spectral methods may not lead to desirable analysis results due to the strong spectral overlapping characteristics of compounds, decreasing signal intensity with worsening signal-to-noise ratio (S/N) in higher derivative orders.

In the analysis method based on the separation technique, the chromatographic methods and their hyphenated versions require a prior treatment step together with some other tedious analytical process for searching the optimal experimental and separation conditions.

VAL and AML is a combination that used to treat high blood pressure (hypertension). In this antihypertensive combination, AML and VAL are named as calcium channel and angiotensin II receptor blockers, respectively. The routine analysis and quality control of the above drug combination before and after commercial formulation is a very important task for the analytical chemistry in drug industry due to the related regulations and human health.

Simultaneous determination of VAL and AML in their combinations was performed by using the analytical methods including the spectrophotometry [1] and chromatographic methods [2,3].

As described above many analytical methods have been applied to the analysis of mixtures or multicomponent pharmaceutical preparations. The disadvantages of both classical spectroscopic and chromatographic approaches require development of new instrumentation techniques or new mathematical approaches to treat analytical data obtained from spectroscopic and chromatographic instrumentations.

One of the promising techniques is wavelet transform [4] and its combination with other classical analysis methods. Particularly, the applications of the wavelet technique in analytical chemistry [5-18] represent a powerful and potential resolution of the spectral methods for the quantitative analysis of compounds in samples.

In this paper two new CWT approaches, db10-CWT and dmey-CWT were improved for the simultaneous quantitative analysis of two-component pharmaceutical preparation containing VAL and AML drugs. These methods do not require the use of additional chemical separation of compounds with each other. The UV- spectra of compounds and their samples were transferred into wavelet domain and processed by db10-CWT and dmey-CWT methods. As a comparison the first derivative spectral transform (DS^1) was used for the same analysis problems consisting of VAL-AML mixtures. After that, the method validation was carried out by using the recovery studies and the standard addition technique with the validation parameters. A good agreement was observed in the application of db10 and dmey-CWT families to the quality control and routine analysis of VAL-AML tablets.

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Experimental

Apparatus and software

A Shimadzu UV-160 double beam UV–VIS spectrophotometer equipped with a fixed slit width (2 nm) was used for the registration of the absorption spectra. The spectrophotometric apparatus was connected to a computer loaded with Shimadzu UVPC software as well as HP Laser Jet P1005. Microsoft Excel together with wavelet toolbox in Matlab 7.0 was used for calibrations and mathematical treatments.

Commercial tablet formulation

A pharmaceutical preparation consisting of film-coated tablets produced by Novartis Pharma Stein AG, Schaffhausertrasse, CH-4332 Stein- Switzerlad was studied. Exforge ® Tablets (Batch No: S0081) contain 10 mg of AML and 160 mg of VAL per tablet. In this study, tablets was analyzed by new spectral signal processing methods, continuous wavelet transforms.

Standard and calibration solutions

Stock solutions were separately prepared by dissolving 25 mg of the compounds, AML and VAL, in 100 mL in methanol. The amount of AML equivalent to 34.7 mg of amlodipine besylate salt was used for the above AML stock solution. Calibration solutions in the linear cocentration range of 2.0-32.0 μg/mL for AML and 2.0-42.0 μg/mL for VAL were prepared by means of the above stock solutions of AML and VAL. An independent validation set or test set containing 12 synthetic mixture solutions of AML and VAL in the above mentioned concentration range for both AML and VAL was prepared, respectively.

Commercial sample preparation

20 tablets were accurately weighed by making use of an electronic balance and tablet content was powdered in a mortar. An amount containing AML and VAL corresponding to one tablet content was dissolved in methanol and in 100 mL calibrated flask. The obtained solution was mechanically shaken for 30 min and filtrated into a 100 mL volumetric flask through a 0.45 μm membrane filter. After that, the solution was diluted by using methanol into calibration concentration range. This procedure was repeated 8 times. The absorption spectra of tablet samples were recorded in the spectral range of 200-305 nm and after that the spectra were stored in computer for the signal processing.

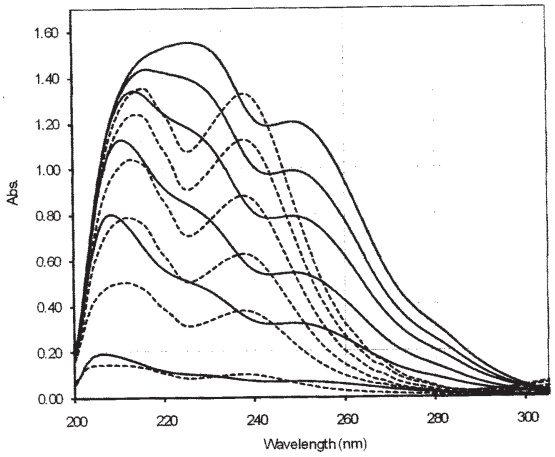

Fig. 1. UV-spectra of the analyzed compounds in the linear concentration range of 2, 10, 18, 26, 34, 42 VAL (—) and 2, 8, 14, 20, 26, 32 AML (- - -) in methanol

Results and discussions

The main issue of the simultaneous spectrophotometric analysis is the overlapping spectral bands of analytes in the same spectral range. As it is known, the classical spectrophotometric method cannot solve this drawback. The aim of this study is to develop new signal processing method based on CWT combined with zero-crossing technique for the simultaneous analysis of AML and VAL in samples without any separation step.

In this paper,the absorption spectra of both compounds, as well as their binary mixture were recorded with the intervals of $\Delta\lambda$ = 0.1 nm in the spectral region of 200-305 nm as it can be seen from figure 1. These spectra were stored in computer to use the following signal processing.

CWT method

For a given mixture analysis the key point is to find within CWT families the one which provides the best recovery and analysis results. Technically, this means that we have to select the optimal wavelet family, namely to identify its scale parameter.

Prior to analysis, the UV spectra of analyzed compounds and their samples were recorded in the above mentioned spectral range. In this study, several continuous wavelet families were tested and we retained db10-CWT and dmey-CWT.

The calibration curves for AML and VAL were obtained by measuring the CWT amplitudes at those points providing zero-crossing. As it can be seen from table 1, for VAL we

	db10-CWT		dmey-CWT		DS[1]	
Parameter	VAL	AML	VAL	AML	VAL	AML
λ (nm)	263.3	270.6	258.5	265.7	287.0	248.9
Range (μg/mL)	2.0-42.0	2.0 -32.0	2.0-42	2.0- 32.0	2.0- 42.0	2.0- 32.0
m	$3.49x10^{-2}$	$3.66x10^{-2}$	1.52E-02	$2.47x10^{-2}$	$9.62x10^{-3}$	$6.18x10^{-2}$
n	$1.17x10^{-1}$	$3.26x10^{-2}$	$2.49x10^{-2}$	$1.69x10^{-2}$	7.11E-03	$4.76x10^{-2}$
r	0.9999	0.9998	0.9994	0.9995	0.9990	0.9992
SE(m)	$5.87x10^{-4}$	$1.11x10^{-3}$	$5.48x10^{-4}$	$8.70x10^{-4}$	$1.23x10^{-4}$	$1.25x10^{-3}$
SE(n)	1.22E-03	2.02E-03	8.00E-04	1.74E-03	5.89E-04	$4.99x10^{-3}$
SE(r)	1.96E-02	2.80E-02	1.83E-02	$1.57x10^{-2}$	$4.12x10^{-3}$	$3.13x10^{-2}$
LOD (μg/mL)	0.26	0.41	0.39	0.52	0.45	0.59
LOQ (μg/mL)	0.86	1.35	1.29	1.73	1.50	1.98

m = Slope of linear regression equation,
n = Intercept of linear regression equation,
r = Correlation coefficient,
SE (m) = Standard error of slope,
SE(n) = Standard error of intercept,
SE(r) = Standard error of Correlation coefficient,
LOD = Limit of detection and LOQ= limit of quantitation

Table 1
LINEAR REGRESSION ANALYSIS AND ITS STATISTICAL RESULTS

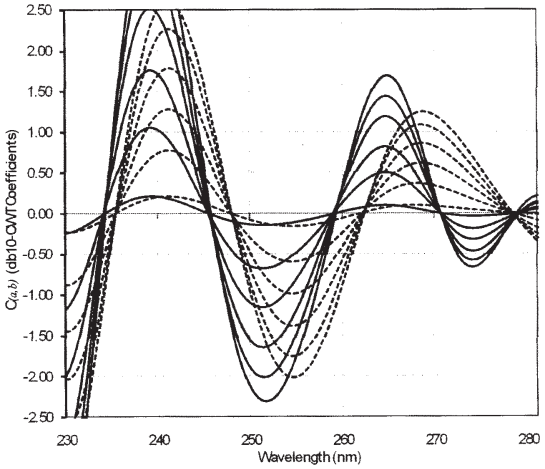

Fig. 2. db10-CWT spectra of the analyzed compounds in the linear concentration range of 2, 10, 18, 26, 34, 42 VAL (—) and 2, 8, 14, 20, 26, 32 AML (- - -) by using db10-CWT (a=140)

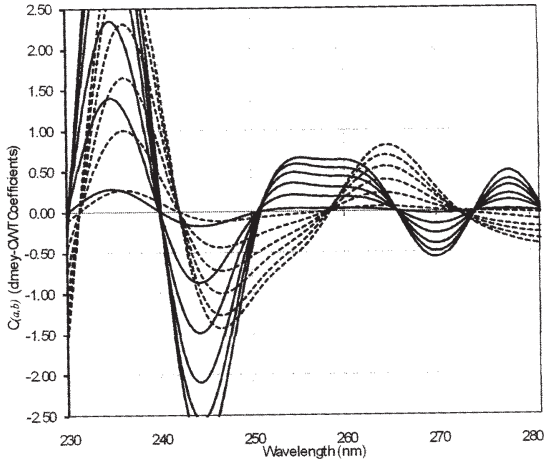

Fig. 3. dmey-CWT spectra of the analyzed compounds in the linear concentration range of 2, 10, 18, 26, 34, 42 VAL (—) and 2, 8, 14, 20, 26, 32 AML (- - -) by using dmey-CWT (a=147)

found 263.3 nm by using db10-CWT, and for AML we obtained 270.6nm for the same wavelet family. For the second wavelet family, namely dmey-CWT, we identify the points 258.5 for VAL and 265.7 for AML, respectively. As another wavelet family, db10-CWT (a=140) was applied to the UV spectra of the analyzed compounds in the linear concentration range of 2-42 μg/mL of VAL and 2- 32 μg/mL of AML as it can be seen from figure 2. Calibration graphs and statistical results by using the linear regression analysis were shown in table 1.

By applying the same methodology and keeping the same concentration range for VAL and AML as described before we obtained the results depicted in figure 3 in the application of dmey-CWT (a=147). Both wavelet families were applied to the UV-spectra in the spectral range between 230-281.1 nm as presented in figures 2 and 3.

The simultaneous quantitative analysis of AML and VAL in their synthetic mixtures and tablets was performed by applying db10-CWT and dmey-CWT to the UV spectra in the presence of the overlapping signal conditions without requiring any chemical separation step.

First derivative spectrophotometry

DS[1] method was applied to the simultaneous determination of AML and VAL in tablets. The absorption UV-spectra of AML and VAL were recorded in the wavelength range 210-350 nm as shown in figure 1. First order derivative of the absorption spectra of two compounds and their samples were calculated with the intervals by using $\Delta\lambda$=5 nm and scaling factor of 5 in the range 200-305 nm selected from the above spectral range.

Figure 4 depicts DS[1] of AML and VAL in the range 200.0-305.0 μg/mL. Calibration graphs were obtained by measuring $dA/d\lambda$ values at 248.9 nm for AML and 287.0 nm for VAL. The statistical results for the linear regression analysis can be found in table 1. The calculated calibration equations were applied to the quantitative analysis of AML and VAL in tablets.

Validity of the methods

In the procedure of the analytical method validation, db10-CWT and dmey CWT give a good linearity in the concentration ranges between 2.0-32.0 μg/mL for AML and 2.0-42.0μg/mL for VAL. Similar linearity for the comparison method (DS[1]) was observed. Correlation coefficient (r) for linearities for the methods is given in table 1. In addition, the recovery study was carried out for the observation of the accuracy and precision of the experimental results obtained by application of the db10-CWT ,dmey-CWT and

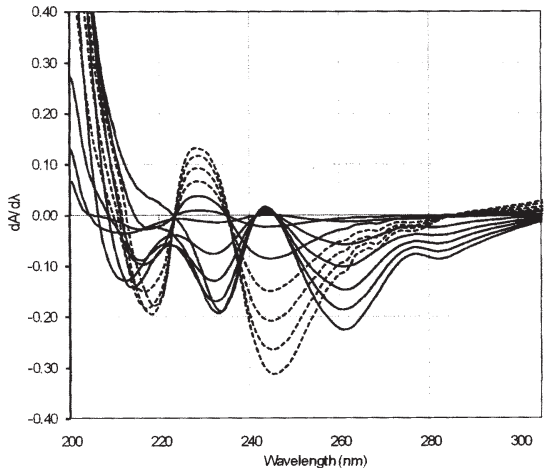

Fig. 4. DS[1] spectra of the UV-signals of the compounds in the linear concentration range of 2, 10, 18, 26, 34, 42 VAL (—) and 2, 8, 14, 20, 26, 32 AML (- - -) by using $\Delta\lambda$=5 nm

DS[1] to the synthetic mixtures containing AML and VAL compounds in the above mentioned concentration range. The percent mean recovery results and their corresponding relative standard deviations (RSD) were illustrated in table 2.

Other validation parameters, the limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed signal processing methods were computed and presented in table 1.

Standard addition technique was used to investigate the selectivity of the proposed signal processing methods. Appropriate volumes of the standard stock solutions of AML and VAL compounds at three different concentration levels, 5.0, 10.0 and 15.0 μg/mL for AML and VAL were added to the tablet solutions. The procedure was repeated six times for each concentration level. The recovery results, standard deviations, relative standard deviations as well as the percent relative error were calculated and depicted in table 3. No interference of the tablet excipients on the determinations was reported during the analysis. By analyzing the recovery studies and the standard addition technique we concluded that the numerical results obtained by db10-CWT and dmey-CWT were in a good agreement with those obtained by DS[1] method.

Analysis of the tablet samples

The proposed CWT methods, db10-CWT and dmey-CWT, were applied for the determination of AML and VAL

Table 2
RECOVERY DATA OBTAINED FROM THE SYNTHETIC BINARY MIXTURES BY USING THE PROPOSED SIGNAL ANALYSIS METHODS

			Calculated amount (μg/mL)						Recovery (%)					
			db10-CWT		dmey-CWT		First derivative		db10-CWT		dmey-CWT		First derivative	
	Binary mix.		263.3	270.6	258.5	265.7	287	248.9	263.3	270.6	258.5	265.7	287	248.9
No.	VAL	AML	VAL	AML	VAL	AML	VAL	AML	VAL	AML	VAL	AML	VAL	AML
1	2.0	2.5	2.01	2.53	2.02	2.51	2.01	2.48	100.3	99.0	101.0	100.5	100.3	99.2
2	10.0	2.5	10.08	2.55	10.36	2.55	9.75	2.50	100.8	98.2	103.6	102.0	97.5	99.9
3	18.0	2.5	17.65	2.54	18.59	2.47	17.80	2.49	98.0	98.6	103.3	98.7	98.9	99.6
4	26.0	2.5	25.85	2.55	26.28	2.51	25.74	2.54	99.4	97.9	101.1	100.3	99.0	101.6
5	34.0	2.5	34.51	2.49	34.27	2.48	34.56	2.60	101.5	100.5	100.8	99.1	101.7	104.0
6	42.0	2.5	41.42	2.52	41.70	2.50	41.83	2.52	98.6	99.4	99.3	100.2	99.6	100.8
7	40.0	2.0	39.25	2.00	40.83	2.03	39.75	2.03	98.1	99.8	102.1	101.4	99.4	101.3
8	40.0	8.0	40.72	7.90	40.38	8.18	41.34	8.24	101.8	101.2	100.9	102.3	103.3	103.0
9	40.0	14.0	40.49	14.29	39.40	13.86	40.76	14.26	101.2	98.0	98.5	99.0	101.9	101.9
10	40.0	20.0	39.14	19.71	39.01	20.13	40.45	20.20	97.9	101.5	97.5	100.7	101.1	101.0
11	40.0	26.0	40.16	26.48	39.68	26.15	41.97	26.65	100.4	98.2	99.2	100.6	104.9	102.5
12	40.0	32.0	39.46	31.65	40.04	32.28	41.53	31.75	98.7	101.1	100.1	100.9	103.8	99.2
								Mean	99.7	99.4	100.6	100.5	101.0	101.2
								SD	1.44	1.34	1.82	1.13	2.25	1.53
								RSD	1.45	1.35	1.81	1.12	2.23	1.51

SD = Standard deviation, RSD = Percent relative standard deviation

Method	Compound	Added (μg/mL)	Mean found (μg/mL)	Mean recovery (%)	SD	RSD
db10-CWT	VAL	5	5.08	101.5	2.27	2.23
		10	10.09	100.9	1.12	1.11
		15	15.25	101.7	1.53	1.51
	AML	5	4.93	98.5	1.63	1.66
		10	10.00	100.0	2.43	2.43
		15	15.03	100.2	1.35	1.35
dmey-CWT	VAL	5	5.05	101.0	0.71	0.70
		10	9.86	98.6	1.24	1.25
		15	14.94	99.6	0.95	0.95
	AML	5	5.05	101.0	0.60	0.60
		10	9.93	99.3	2.36	2.38
		15	15.12	100.8	0.88	0.88
DS[1]	VAL	5	4.99	99.8	2.47	2.47
		10	10.17	101.7	1.48	1.46
		15	15.17	101.1	2.21	2.19
	AML	5	5.03	100.7	1.54	1.53
		10	9.71	97.1	2.39	2.46
		15	15.41	102.7	2.40	2.33

Results were obtained from the average of 5 replicates

Table 3
RECOVERY DATA AND STATISTICAL RESULTS FOR THE STANDARD ADDITION TECHNIQUE

in commercial pharmaceutical tablets. The comparison DS[1] method was used for the quantitative analysis of tablets. The results obtained are illustrated in table 4 and they show that the tablet determination results are satisfactory in all cases. Before tablet analysis the standard addition technique was applied to observe the effect of the excipients on the tablet analysis. Any interference of excipients was not observed in the application of the proposed methods to tablet analysis as well as the application of the standard addition technique.

In this work VAL and AML give the overlapping spectra in the spectral range between 200-305 nm as it can be seen from figure 1. For this reason the determination of two compounds in the same mixture is impossible by using the traditional spectroscopic methods. As it is known the analysis of binary mixtures requires a priori separation step. To find a simple analytical resolution of overlapping spectra and to eliminate the priori separation step of chemicals a new and powerful wavelet tool was proposed for the simultaneous quantitative analysis of AML and VAL in their synthetic mixtures and tablets. In this context two wavelet families' db10-CWT and dmey-CWT combined with zero-crossing technique give successful determination results. In this study the classical DS[1] was applied to the analysis of tablets consisting of AML and VAL and it was observed

Table 4
TABLET DETERMINATION RESULTS BY APPLYING SIGNAL PROCESSING METHODS

	mg/tablet					
	db10-CWT		dmey-CWT		DS[1]	
	VAL	AML	VAL	AML	VAL	AML
No.	**263.3**	**270.6**	**258.5**	**265.7**	**287.0**	**248.9**
1	159.7	9.9	158.0	10.0	155.3	9.6
2	160.3	10.2	159.2	10.0	156.5	9.7
3	159.0	10.0	156.1	10.3	154.1	9.6
4	159.7	10.0	156.2	10.3	156.2	9.7
5	157.8	9.9	158.7	10.3	151.7	9.8
6	158.0	10.1	161.8	10.0	157.7	9.7
7	157.4	9.8	160.6	10.3	161.1	9.8
8	158.5	10.2	157.8	10.0	157.0	10.1
Mean	158.8	10.0	158.5	10.1	156.2	9.8
SD	1.05	0.14	2.01	0.15	2.74	0.17
RSD	0.66	1.36	1.27	1.48	1.75	1.69
SE	0.37	0.05	0.71	0.05	0.97	0.06
CL	0.73	0.09	1.39	0.10	1.90	0.11

SE = Standard error
CL = Confidential level (p = 0.05)
Claimed label of one commercial tablet = 160 mg VAL and 10 mg AML

that we obtained small derivative amplitude; therefore we were forced to use a scale factor to get rid of this drawback. The process of finding the optimal scale factor is not an easy task for a given complex mixture, as the one investigated in this study, particularly it is time consuming. On the contrary the wavelet analysis, due to its mathematical properties, can be easily applied to complex mixture and the results can be obtained in a shorter period of time. Therefore, two new wavelet families, db10-CWT and dmey-CWT , were found suitable to be used for the quality control and routine analysis of AML and VAL drugs in tablets.

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