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Continuous wavelet and derivative transforms for the simultaneous quantitative analysis and dissolution test of levodopa–benserazide tablets

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Abstract

Simultaneous analyses and dissolution tests of levodopa–benserazide tablets were carried out by continuous wavelet transform (CWT) and classic derivative spectrophotometry (DS) without using any chemical separation step. The developed two spectrophotometric resolutions are based on the transformation of the original UV spectra. The original absorption spectra of levodopa and benserazide in the concentration range of 1–80 μg/mL and 5–240 μg/mL in USP simulated gastric juice were registered in the spectral range of 250–310 nm, respectively. Various wavelet families and different spectrophotometric derivative orders were tested to find the optimal signal processing for obtaining desirable calibration graphs and reliable determinations of the investigated drugs. Under the optimized conditions of the methods, symlets wavelet family using $a = 128$ with sixth order (SYM6–CWT) and the first derivative transform with $\Delta\lambda = 10$ nm were identified as optimal signal processing methods for the determinations and dissolution tests. The calibration functions for each drug were obtained by measuring the values of the CWT and derivative amplitudes. The validation of the developed methods was confirmed by analyzing various synthetic mixtures of the investigated drugs. Mean recovery values were found between 99.1% and 104.7% for DS and 100% and 102.9% for CWT, respectively for determination of BEN and LEV in synthetic mixtures. Each developed approaches were successfully applied to the simultaneous determination and dissolution test of levodopa and benserazide in their commercial tablets and a good agreement was observed.

Keywords: Dissolution test; Levodopa; Benserazide; Continuous wavelet transform; Derivative transform

1. Introduction

Antiparkinsonian drug levedopa (LEV), a dopamine precursor, is often combined with an amino acid decarboxylase inhibitor such as carbidopa (as in Sinemet) or benserazide (BEN) (as in Madopar). The proportion of LEV and BEN in commercial dosage forms is 4:1. The chemical structures of these two drugs are shown in Fig. 1.

Simultaneous determination of LEV and BEN in their mixtures were performed by several analytical methods, e.g., HPLC [1], spectrophotometry [2], multivariate calibration [3], capillary electrophoresis [4], kinetic spectrophotometric technique [5], partial least squares calibration [6]. The official methods for the simultaneous determination of LEV and BEN with its impurity (*R*,*S*)-2-amino-3-hydroxipropanohydrazide were used in British Pharmacopoeia [7,8].

Simultaneous dissolution tests on some drugs in combined pharmaceutical formulations have been achieved by various spectrophotometric analytical methods [9–12].

Recently, continuous wavelet transform (CWT) method plays an important role in signal processing technique for the overlap-

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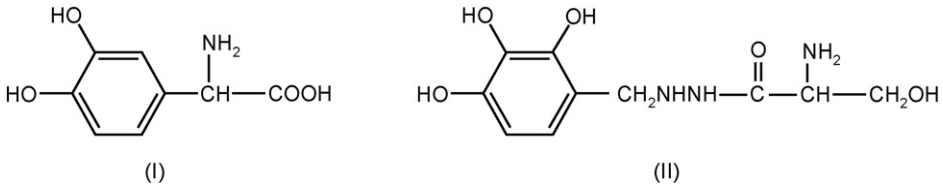

Fig. 1. Molecular structures of LEV (I) and BEN (II) drugs.

ping peak resolution and for the significant peak identification. The wavelet transform and its applications in analytical chemistry and its neighborhood branches have been reported for the simultaneous determination of drugs in multicomponent pharmaceutical preparations [13–19].

In this study, two new graphical approaches, CWT and derivative spectrophotometry (DS) were developed for the simultaneous determination and dissolution tests of LEV and BEN in tablets. The effect of the oxidation of BEN in the aqueous solution at higher than pH 3–4, during the analysis procedure was eliminated by the simultaneous use of dark and red light environment, the preparation of the test solutions having SGJ at pH 1.2, the usage of deaired SGJ and the argon gas passed through.

The developed analytical approaches were tested by analyzing several synthetic mixtures consisting of LEV and BEN and by considering the standard addition technique. The comparative analysis of the results delivered by the above-investigated analytical approaches show a good agreement between the obtained results.

2. Materials and methods

2.1. Apparatus and software

The absorption spectra were recorded by using a Shimadzu UV-160 double beam UV–vis spectrophotometer having a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK-E320 printer. The spectrum was recorded in the wavelength range of 250–310 nm against a blank simulated gastric juice [SGJ (USP XXVIII)]. Aymes 6 model was used as a dissolution test apparatus.

The data treatment was performed in a Pentium 4, 2.8 GHz (512 MB RAM) computer using MATLAB 7.0 software (Math Works, Natick, MA, USA) and calculations and calibrations were done using Microsoft EXCEL.

2.2. Reagents

Hydrochloric acid (Merck), sodium chloride (Merck) and Madopar® 125 Tablets (Roche Ind. Pharm., Istanbul, Turkey) containing 100 mg of LEV and 25 mg of BEN were used in this study. LEV and BEN were kindly obtained from Roche.

2.3. Preparation of SGJ and light-protected area

SGJ was deaired by heating to 65 °C and then vacuum was applied. After cooling, the media argon gas was passed through. All the test solvents (SGJ), solutions and test place were protected from light and all of the test procedures were carried out in red light and the whole laboratory was lighted with a 15 W red light bulb.

2.4. Standard solution

Stock solutions of 25 mg/100 mL LEV and BEN for each compound were prepared in SGJ. Two standard series solutions containing 1–80 μg/mL LEV and 5–240 μg/mL BEN were obtained by using the stock solutions for the registration of the absorption spectra. A validation set of 20 mixture solutions containing two drugs was prepared by using the same stock solutions.

2.5. Commercial tablets analysis

Twenty tablets were accurately weighed and powdered in a mortar. A sample containing LEV and BEN equivalent to a tablet content was dissolved in 1000 mL SGJ. The content of the flask was mechanically shaken for a period of 30 min and filtered with 0.20 μm disposable membrane filter (Sartorious, minisart, $\varphi = 0.20$ μm) with the help of a syringe. After that the developed methods were applied to the final solution ($n = 10$).

2.6. Dissolution test of tablet

Dissolution test was carried out by USP paddle method with 50 rpm in SGJ at 37 °C. The sample was taken by means of a syringe with membrane filter (0.20 μm) and diluted with sufficient quantity of dissolution medium. The final solution ($n = 6$) was analyzed by the analytical methods presented in this study. Before the sample was taken, the membrane filter was saturated with the active ingredients in order to avoid possible adsorption onto the membrane filter at the beginning of the test by discharging the sample into the vessel three times—the fourth sample was being used. After dissolution media (SGJ) was deaired, argon gas passed through for 1 min at the beginning of the test and then argon gas passed through the dissolution medium for 1 min for a time interval of 15 min during the solution test.

2.7. Wavelet method

This approach was successfully applied to the spectrophotometric multicomponent analysis of relevant compounds in samples.

Let us choose a mother wavelet [20,21] by $\Psi(\lambda)$. By using scaling and shifting on it, a set of functions denoted by $\Psi_{a,b}(\lambda)$

is obtained as follows

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

where a denotes the variable scale parameter, used to control the scaling and b is the translation parameter controlling the translation and R is the domain of real numbers.

Therefore, CWT of a given signal denoted by $f(\lambda)$ is defined by the following formula:

$$\mathrm{CWT}\{f(\lambda); a, b\} = \int_{-\infty}^{\infty} f(\lambda)\ \ \psi^*_{a,b}(\lambda)\,\mathrm{d}\lambda = \left\langle f(\lambda), \psi_{a,b}\right\rangle \tag{2}$$

The superscript '*' denotes the complex conjugate and $\left\langle f(\lambda), \psi_{a,b}\right\rangle$ is the inner product of function $f(\lambda)$ onto the wavelet function $\Psi_{a,b}(\lambda)$. In this study we used the wavelet family sym6.

3. Results and discussion

3.1. Method development

The developed methods are based on the continuous wavelet and derivative transforms of the absorption spectra obtained from investigated BEN and LEV drugs. As it is depicted in Fig. 2a, the absorption spectra of both drugs strongly overlap in the spectral region of 250–310 nm. Due to their mutual interference, the simultaneous determination of LEV and BEN in the same samples is not possible by using the classical analytical methodologies. On the other hand, the simultaneous dissolution tests of the subjected two drugs is also not possible due to the above-presented reasons.

To solve the above-mentioned problems, CWT and DS approaches were developed and successfully applied to the simultaneous analysis and dissolution tests of tablets containing both LEV and BEN.

Amino acid decarboxylase inhibitor BEN is very sensitive to oxidation in aqueous solution, especially above pH 3–4. BEN can be easily oxidized in the presence of oxygen dissolved in water and light can accelerate this reaction. Using low pH aqueous solution (SGJ), deairation and then argon gas pass through and lighting the laboratory with red bulb, are found to be enough to avoid the oxidation during tests.

3.2. CWT method

Absorption spectra of BEN and LEV in the linear concentration range of 5.0–240 μg/mL and 1–80 μg/mL, respectively were recorded in the spectral range of 250–310 nm and are illustrated in Fig. 2a. CWT sym6 ($a = 128$) was applied on the absorption spectra and the graphs of CWT are presented in Fig. 2b. Calibration functions of BEN and LEV were obtained by measuring the CWT signals at 273 and 282.9 nm, respectively. Linear regression analysis and its statistical results are shown in Table 1. Validation of the method was carried out by analyzing the synthetic mixtures of both drugs and a good agreement was reported. Recovery results and their statistical results were shown in Table 2.

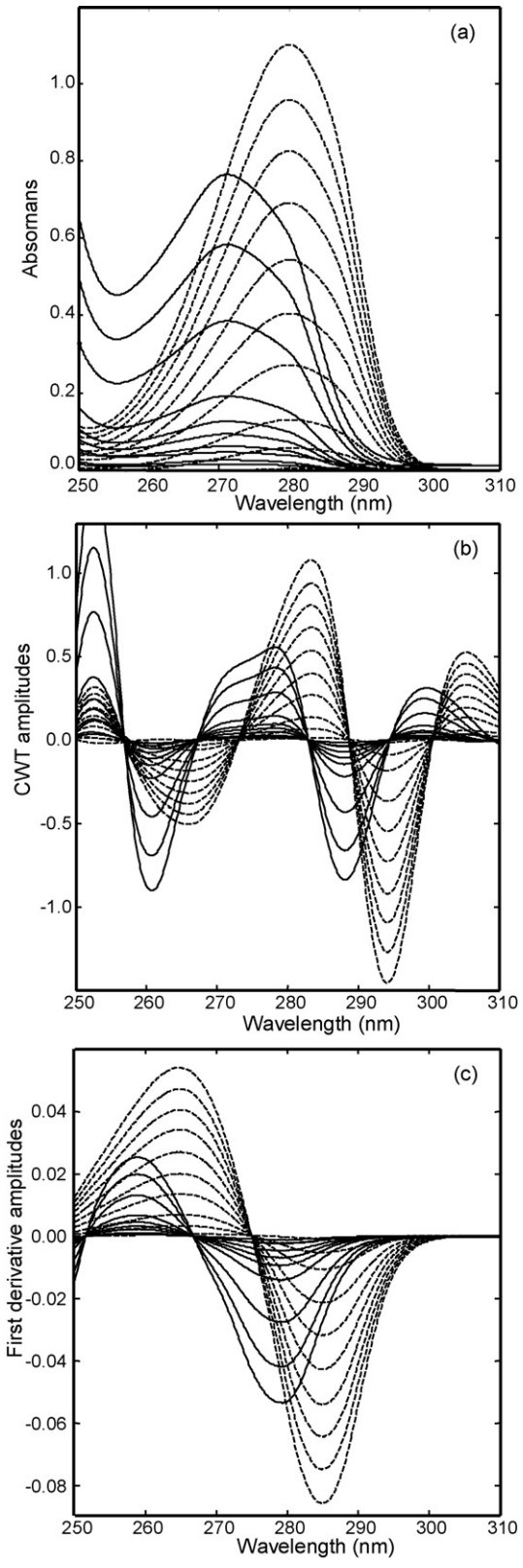

Fig. 2. (a) Absorption spectra, (b) CWT spectra and (c) first derivative spectra of BEN (—) and LEV (- - -) in the concentration range of 5–240 μg/mL and 1–80 μg/mL, respectively, in gastric juice (USP XXVIII).

Table 1
Linear regression analysis and its statistical results

	CWT[a]		DS[a]	
	BEN[b] 273.0[c]	LEV[b] 282.9[c]	BEN[b] 275.0[c]	LEV[b] 266.8[c]
Range (μg/mL)	5–240	1–80	5–240	1–80
m	1.89×10^{-3}	1.34×10^{-2}	1.81×10^{-4}	6.51×10^{-4}
n	2.81×10^{-4}	-1.30×10^{-3}	-6.68×10^{-5}	-8.51×10^{-5}
r	0.9997	0.9999	0.9995	0.9999
LOD (μg/mL)	0.25	0.64	0.83	0.28
LOQ (μg/mL)	0.84	2.12	2.77	0.92

r, correlation coefficient of the regression function; *m*, slope of the regression function; *n*, intercept of the regression function; *r*(S.E.), standard error of the correlation coefficient; *m*(S.E.), standard error of the slope; *n*(S.E.), standard error of the intercept; LOD, limit of detection; LOQ, limit of quantitation.
[a] Method
[b] Drug
[c] λ (nm)

3.3. DS method

In the classical derivative method, the absorption spectra of LEV and BEN in the linear concentration range of 1–80 μg/mL and 5–240 μg/mL, respectively, were plotted in the wavelength range of 250–310 nm (as it can be seen in Fig. 2a). First derivative of the absorption spectra of two drugs and their samples were calculated by using the interval of $\Delta\lambda = 10$ nm as shown in Fig. 2c. Linear regression functions for LEV and BEN compounds were obtained by measuring the $dA/d\lambda$ values at 275.0 and 266.8 nm corresponding to the zero-crossing points, respectively. Linear regression analysis and its statistical results were presented in Table 1. As it can be seen from this table, satisfactory results were obtained. Regression functions obtained were applied to the simultaneous quantitative evaluation of LEV and BEN in their samples.

To check the effect of excipients on the quantitative analysis, the standard addition technique was applied. The recoveries and relative standard deviations of standard addition technique were reported as 99.6% with 2.37% for BEN and 99.5% with 1.80% for LEV by using CWT and 97.6% with 3.11% for BEN and 99.6% with 2.39% for LEV by using DS. The above-mentioned results were obtained by using five replicates for five different concentration levels. No interference of excipients was observed during analysis.

The CWT and DS methods were applied to the commercial sample containing BEN and LEV and the obtained results were presented in Table 3. A good coincidence was observed for the assay results with the declared amount of the components on the label.

3.4. Method validation

A good linearity for BEN and LEV in the range of 5–240 μg/mL and 1–80 μg/mL, respectively was observed according to the correlation coefficients (*r*) as presented in Table 1.

Precision of the developed CWT and DS approaches was evaluated by analyzing 20 solutions consisting of BEN–LEV mixtures by dilution of the stock solution.

R.S.D. was calculated by using the following formula

$$\%\ \text{R.S.D.} = \frac{\text{S.D.} \times 100}{X} \quad (3)$$

where, S.D. represents the standard deviation and *X* denotes the average of the percentage main recovery. The percentage recoveries and R.S.D. values corresponding to the precision were presented in Table 2.

The limit of detection (signal-to-noise ratio of 3:1) and the limit of quantitation (signal-to-noise ratio of 10:1) were calculated by using the data obtained from six replicates for standard

Table 2
Recovery data obtained by application of the developed methods to the synthetic mixtures

Added (μg/mL)		Recovery (%)			
		CWT		DS	
BEN	LEV	BEN	LEV	BEN	LEV
15.0	1.0	101.5	96.2	100.4	94.0
15.0	5.0	100.1	100.6	100.1	95.7
15.0	10.0	99.6	102.4	100.4	94.0
15.0	20.0	104.8	101.2	104.1	99.9
15.0	30.0	98.3	100.7	101.1	99.9
15.0	40.0	103.5	100.5	101.1	100.3
15.0	50.0	104.7	100.5	101.1	99.9
15.0	60.0	103.8	101.2	104.8	100.1
15.0	70.0	97.8	100.5	104.8	98.0
15.0	80.0	103.2	99.7	104.8	97.6
5.0	60.0	100.8	100.7	105.9	99.5
10.0	60.0	102.5	101.5	102.1	101.6
15.0	60.0	104.9	100.6	115.1	101.4
20.0	60.0	108.5	100.4	108.4	101.1
30.0	60.0	106.3	99.7	107.2	100.4
40.0	60.0	106.8	100.4	106.6	99.9
60.0	60.0	99.7	101.5	105.0	100.4
120.0	60.0	102.9	100.8	107.6	100.4
180.0	60.0	103.1	97.6	105.4	99.3
240.0	60.0	104.2	97.1	107.6	98.3
Mean		102.9	100.2	104.7	99.1
S.D.		2.85	1.53	3.66	2.21
R.S.D.		2.77	1.53	3.50	2.23

S.D., standard deviation. R.S.D., relative standard deviation.

Table 3
Determination results of BEN and LEV drugs by the developed methods

mg/tablet				
No.	CWT		DS	
	BEN	LEV	BEN	LEV
Mean	25.3	96.7	25.9	96.7
S.D.	0.64	1.93	0.95	1.90
R.S.D.	2.52	1.99	3.68	1.97
SE	0.20	0.61	0.30	0.60
CL ($p = 0.05$)	0.14	0.41	0.20	0.41

CL, confidence limit.

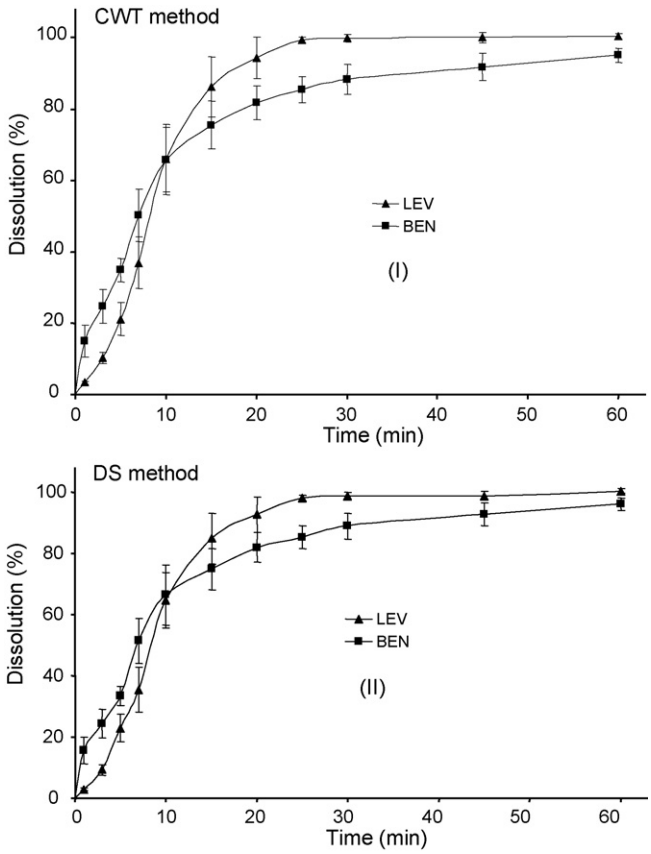

Fig. 3. Dissolution profiles of BEN and LEV for six combined commercial tablets obtained by CWT (I) and S (II) methods.

solutions of BEN at 15 μg/mL and LEV at 60 μg/mL. The intercept and slope values of calibration function and their values are shown in Table 1.

To investigate the effect of tablet excipients on the analysis, the standard addition technique was applied to the samples obtained by mixing pure components and their commercial forms and their recovery results were presented in the above section. According to the obtained results, no effect of the excipients on the analysis was observed.

3.5. Tablet analysis

The quantitative analysis of BEN and LEV drugs in commercial tablets were carried out by CWT and DS approaches. The assay results of pharmaceutical tablet formulation are shown in Table 3. It was observed that the determination results are very close to each other; therefore a good agreement was reported.

3.6. Dissolution test

Dissolution data and dissolution profiles of the commercial tablets consisting of LEV and BEN by CWT and DS approaches were shown in Fig. 3(I) and (II), respectively. Approximately 65% of both drugs were dissolved within the first 10 min. The dissolution percentage and the confidential limit ($p = 0.05$) were found to be as 99.9 and 1.08 for LEV by CWT and 98.76 and 2.53 for LEV by DS at the 45th min. The numerical results for BEN at the 60th min are 95.0 for the dissolution percentage and 1.93 for confidential limit using CWT and 96.0 for the dissolution percentage and 2.25 for the confidential limit using DS approach, respectively.

4. Conclusions

Although the absorption spectra of BEN and LEV overlap in the same spectral range 250–340 nm, the simultaneous analysis and dissolution test of BEN–LEV tablets were successfully performed by the proposed CWT and DS approaches. Both CWT and DS methods are simple, sensitive, easy to apply and very cheap for routine analysis, quality control of LEV and BEN as well as the determination of drug-dissolution profiles. In this study, the CWT and DS methods were successfully applied to obtain the simultaneous dissolution profiles of BEN and LEV in tablets without any prior separation procedure. Results showed that these two new approaches can be applied to the routine quality control of the multicomponent dosage forms containing BEN and LEV.

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