A Signal Processing Tool Based on the Continuous Wavelet for the Simultaneous Determination of Estradiol Valerate and Cyproterone Acetate in their Mixtures

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A signal processing tool based on the continuous wavelet transform (CWT) was proposed for the simultaneous determination of estradiol valerate (EV) and cyproterone acetate (CA) in their mixtures. Different continuous wavelet families were tested in order to find the optimum family of the wavelets. The Symlets 8 continuous wavelet transform (SYM8-CWT) was observed to be the optimal one. The SYM8-CWT approach was applied to the absorption spectra of the related compounds in the spectral range of 210.00-312.35 nm for the calibration solutions of the compounds in the concentration range of 10-50 µg/mL for EV and 5-30 µg/mL for CA. The calibration equations for EA and CA were computed by measuring the continuous wavelet signals at 260.75 nm and 238.40 nm, respectively. The proposed method was validated by the independent analysis of the synthetic mixtures and by the standard addition technique. It was observed that the selectivity and the sensitivity in the application of the db5-CWT method to the determination of EV and CA compounds were satisfactory.

Keywords: Continuous wavelet transforms, simultaneous determination, estradiol valerate, cyproterone acetate, binary mixture

Combination of estradiol valerate (EV) with cyproterone acetate (CA) is used in hormone replacement therapy during climacterium. Estradiol valerate and cyproterone acetate are completely absorbed after oral administration. During the absorption process and the first liver passage, estradiol valerate but not cyproterone acetate is extensively metabolised. Despite complete absorption and ester hydrolysis of estradiol valerate only 3% of the dose is bioavailable as estradiol after oral administration. Cyproterone acetate is completely bioavailable after oral administration. During the absorption process and the first liver passage estradiol valerate is rapidly hydrolysed to 17beta-estradiol and valeric acid. Cyproterone acetate is mainly metabolised to 15beta-OH cyproterone acetate, a pharmacologically active metabolite with similar high antiandrogenic but much lower progestogenic activity as compared to the parent drug [1].

Ratio derivative spectrophotometric and chemometric methods for the simultaneous quantitative analysis of the commercial preparation samples containing EA and CA were recently developed [2]. On the other hand, several analytical methods including spectrophotometry [3-5], HPLC [6,7], fluorimetry [8] and radioimmunoassay [9] were devoted for the determination of EV and CA in pharmaceutical preparations as well as in the biological fluids. In addition, derivative spectrophotometric method for determination of estradiol valerate in tablets was reported [10].

In this manuscript, a new signal processing method based on the combined use of CWT with zero-crossing technique was proposed for the simultaneous determination of EV and CA in mixtures and in commercially formulation. The proposed SYM8-CWT method was validated by analyzing the synthetic mixtures of EV and CA and by using the standard addition technique. Good agreements for the determination results for the related compounds were reported.

Experimental part

Methods and equipment

The wavelet method

Wavelet method becomes very powerful in many branches of science and engineering [12-13]. Wavelet method is devoted to analyze the signal in time-frequency scale and it is useful in non-stationary signals analysis. In the last decade, the huge potential application of this powerful technique in chemistry especially in combination to other methods leads us to a conclusion that the wavelet method becomes a powerful technique for the quantitative determination of compounds in samples.

In the following we consider a wavelet family denoted by ∅(λ) [12-13]. By making scaling and shifting of ∅(λ) we immediately obtain a set of functions denoted as ∅a,b(λ) and possessing the following form:

$$ψ_{a,b}(λ) = \frac{1}{\sqrt{|a|}} ψ\left(\frac{λ-b}{a}\right), \quad a ≠ 0, \quad a, b ∈ R,$$

(1)

where a represents the scale parameter, b denotes the translation parameter and R is the domain of real numbers. Let's consider a signal f(λ) ∈ L^2(R), and define the continuous wavelet transform as:

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where the superscript * denotes the complex conjugate and \( \langle \psi_{a,b}(\lambda), f(\lambda) \rangle \) means the inner product of function \( f(\lambda) \) onto the wavelet function \( \psi_{a,b}(\lambda) \).

Shimadzu UV-2550 UV-Vis double beam spectrophotometer connected to a computer loaded with Shimadzu UVProbe was used for all the spectrophotometric measurements. The absorption data were transformed into Excel and was processed by SYM8-CWT. Wavelet toolbox in Matlab 7.0 was used for the absorption data transformations.

**Standard solutions**

Solutions of 20 mg/100 mL of CA and EV were prepared in methanol. The calibration solutions of the compounds in the concentration range of 10-50 \( \mu \)g/mL for EV and 5-30 \( \mu \)g/mL for CA were prepared by using the above stock solutions. An independent validation set solutions in the above concentration ranges were prepared from the stock solutions. The standard addition samples were prepared by adding stock solution to the commercial samples.

**Sample preparation**

In the method application, 20 sugar-coated tablets were accurately weighed and powdered in a mortar. An amount of the tablet mass corresponding to one tablet content was dissolved in 25 of methanol. After 30 min of mechanically shaking, the solution was filtered by using a membrane filter (Sartorius Minisart \( \phi = 0.45 \) \( \mu \)m). The SYM8-CWT method was applied to the final diluted solution.

**Commercial preparation**

A commercial preparation (CLIMEN® Sugar-coated tablet, Bayer Schering Pharma, Turkey) was assayed. Its declared content was as follows: 2.0 mg EV and 1.0 mg CA per tablet. CA and EV were donated by the Turkish national Pharm. Ind. firms, Turkey.

**Results and discussions**

The UV absorption spectra of the standard series and samples solutions in the concentration range of 10-50 for EV and 5-30 \( \mu \)g/mL for CA were plotted in the wavelength range of 210-330 nm (fig. 1). The UV absorption spectra data corresponding to 210.00-312.35 nm were selected and transferred to the wavelet domain. The obtained continuous wavelet signals were applied to all wavelet families available on Wavelet Toolbox in the Matlab 7.0 software and the optimal family was retained. As a result SYM8-CWT (a = 1150) was found to be the optimal CWT tool. The obtained wavelet coefficients were plotted versus wavelengths and then SYM8-CWT spectra were obtained as shown in figure 2. The analytical CWT amplitudes at 260.75 nm for EV and for CA at 238.40 nm were measured. Calibration graphs were calculated by using the linear regression analysis based on the relationship between their CWT peak intensity and concentration. The obtained calibration graphs and their statistical results from linear regression analysis were presented in table 1. The results showed high correlation coefficients (r) and satisfactory slope, intercept, the limit of detection and the limit of quantitation obtained by the application of the SYM8-CWT with the selected optimal zero crossing points. This SYM8-CWT method was applied to the simultaneous quantitative analysis of the samples in the above mentioned optimized experimental conditions.

**Validation of SYM8-CWT method**

The linearity of the calibration graphs obtained by applying the SYM8-CWT to the analysis of EV and CA was verified by high correlation coefficients (r) as shown in table 1. In recovery studies, the analysis of 19 samples consisting of the EV-CA combinations in different concentration levels was used to test the precision and accuracy of the proposed wavelet methods. The recovery results with standard deviation and relative standard deviations were indicated in table 2. A good coincidence of the obtained experimental results was observed for the validation of the methods. No degradation product, interference and systematical errors were observed during
the analysis procedure. The standard addition technique was applied to observe the matrix effect or the effect of excipients on the analysis of commercial samples. The corresponding standard addition plots for both compounds were presented in figure 3. We concluded that the slope values of the standard addition and calibration samples became close to each other. As it can be seen in figure 3, no matrix effect was reported during the analysis. The limit of detection (LOD; signal-to-noise ratio of 3:1) and the limit of quantitation (LOQ; signal-to noise ratio of 10:1) were calculated for EV and CA compounds. The results can be seen in table 1.

**Commercial sample analysis**

Quantitative analysis was carried out by applying the SYM8-CWT method to the commercial preparation containing EV and CA. The determination results were shown in table 3. The experimental results and label claim of tablets showed good coincidence. The numerical data for the analysis results of related compounds are acceptable determination limits in application of the proposed method to the tablets.

**Conclusions**

In this paper, the wavelet method was applied for the simultaneous determination of EV and CA in mixtures. This approach was found suitable for the quality control and routine analysis of the pharmaceutical preparation containing EA and CA.

**Acknowledgements**

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**References**

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**Table 3**

**DETERMINATION RESULTS OBTAINED APPLYING THE SYM8-CWT METHOD TO THE VALIDATION SAMPLES**

<table>
<thead>
<tr>
<th>No.</th>
<th>EV (mg/tablet)</th>
<th>SA (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.96</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>2.01</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>2.04</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>2.02</td>
<td>0.98</td>
</tr>
<tr>
<td>6</td>
<td>2.06</td>
<td>1.04</td>
</tr>
<tr>
<td>7</td>
<td>2.01</td>
<td>0.99</td>
</tr>
<tr>
<td>8</td>
<td>2.05</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Declared content of the commercial sugar-coated tablets was as follows: 2.0 mg EV and 1.0 mg CA per tablet

**Table 2**

**RECOVERY DATA OBTAINED BY APPLYING SYM8-CWT METHOD TO THE VALIDATION SAMPLES**

<table>
<thead>
<tr>
<th>No.</th>
<th>Binary mixture (µg/mL)</th>
<th>Found (µg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EV 10, SA 20</td>
<td>9.9, 20.4</td>
<td>99.1, 102.2</td>
</tr>
<tr>
<td>2</td>
<td>EV 15, SA 20</td>
<td>15.6, 20.1</td>
<td>103.9, 100.3</td>
</tr>
<tr>
<td>3</td>
<td>EV 20, SA 20</td>
<td>19.7, 20.5</td>
<td>98.5, 102.5</td>
</tr>
<tr>
<td>4</td>
<td>EV 25, SA 20</td>
<td>24.1, 20.2</td>
<td>96.4, 100.8</td>
</tr>
<tr>
<td>5</td>
<td>EV 30, SA 20</td>
<td>29.5, 19.4</td>
<td>98.3, 97.2</td>
</tr>
<tr>
<td>6</td>
<td>EV 35, SA 20</td>
<td>35.0, 21.0</td>
<td>100.0, 105.1</td>
</tr>
<tr>
<td>7</td>
<td>EV 40, SA 5</td>
<td>39.4, 5.0</td>
<td>98.4, 99.1</td>
</tr>
<tr>
<td>8</td>
<td>EV 40, SA 10</td>
<td>39.1, 10.0</td>
<td>97.8, 99.7</td>
</tr>
<tr>
<td>9</td>
<td>EV 40, SA 15</td>
<td>40.7, 15.2</td>
<td>101.7, 101.2</td>
</tr>
<tr>
<td>10</td>
<td>EV 40, SA 20</td>
<td>39.4, 19.3</td>
<td>98.5, 96.6</td>
</tr>
<tr>
<td>11</td>
<td>EV 40, SA 25</td>
<td>39.6, 25.6</td>
<td>98.9, 102.2</td>
</tr>
<tr>
<td>12</td>
<td>EV 40, SA 30</td>
<td>39.1, 30.0</td>
<td>97.8, 100.0</td>
</tr>
</tbody>
</table>

Mean: 99.1, 100.6
SD: 2.34
RSD: 2.33

**SD** = Standard deviation, **RSD** = Relative standard deviation

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