Application of Continuous Wavelet Transform Coupled with
Zero-crossing Technique for the Simultaneous Spectrophotometric
Determination of Sacubitril and Valsartan in Tablet Dosage Form

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ABSTRACT: A rapid, simple, precise, accurate, and environmentally friendly spectrophotometric method was
developed and validated for simultaneous determination of Sacubitril and Valsartan in their combined dosage form,
using continuous wavelet transform (CWT) and zero-crossing techniques without using organic solvents and the time-
consuming extraction step. Initially, UV spectra of two pure components in water were processed via various mother
wavelet families. Then, applying zero-crossing technique, the optimum points were found to obtain appropriate
calibration curves for each point. The calibration curves were linear for both Sacubitril and Valsartan. The validation
of these methods was investigated by analyzing several synthetic mixtures with known concentrations. Applying one-
way analysis of variance (ANOVA) test and Fisher pairwise comparisons, the following were found to yield the best
results: Discrete Meyer (dmey) wavelet functions with scaling factor of 61 at 232 nm and Symlet5 (sym5) with 48 at
232 nm for Sacubitril and Meyer (meyr) with 50 at 272 nm, meyr with 59 at 247 nm, Daubechies (db5) with 53
at 237 nm, and sym5 with 59 at 226 nm for Valsartan. The mean recovery values in synthetic mixtures were
between 99.09% and 101.16% using the proposed methods, where relative standard deviation (RSD) not more than 1.23%
proved to have good precision. The obtained results from the commercial tablets, applying the developed methods, were
compared to those yielded by HPLC method by one-way ANOVA test. According to the results, they were in good
agreement and showed no significant differences, thereby suggesting successful determination in accordance with
green chemistry.

INTRODUCTION

Presently, deaths related to chronic heart failure with reduced ejection fraction is an important thing that public
health specialists are concerned about [1, 2], because this failure is growing epidemically [3]. This disease occurs
when the muscles of heart become weak and as a result, cannot pump an adequate volume of blood [4]. Angiotensin
receptor–neprilsin inhibition can reduce the risk of death in patients with this failure [5]. A novel drug, consisting of
the nepriysin inhibitor Sacubitril (SAC) and the angiotensin-receptor blocker Valsartan (VAL), is the first
medicine in this category. This combined drug (previously known as LCZ696) is also a useful antihypertensive drug
[6-9]. This new drug was discovered and developed as tablet dosage form called ENTRESTO by Novartis, which

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was then approved by US Food and Drug Administration (FDA) in July 2015 [10]. ENTRESTO is a combined dosage form containing anionic forms of SAC and VAL, sodium cations, and water molecules with the molar ratio of 1:1:3:2.5, respectively which dissociates into SAC and VAL after oral administration. Chemically, this compound is Octadecasodiumhexakis(4-[[1S,3R]-1-[(1,1'-biphenyl)-4-ylmethyl]-4-ethoxy-3-methyl-4-oxobutyl]amino)-4-oxobutanoate)hexakis[N-pentanoyl-N-[(2’-(1H-tetrazol-1-id-5-yl)]1,1'-biphenyl]-4-yl]methyl]-L-valinate—water (1/15) [11]. The chemical structures of LCZ696, SAC, and VAL are illustrated in Figure 1 [12].

![Chemical structures of LCZ696, SAC, and VAL.](image)

**Figure 1.** Chemical structures of LCZ696, SAC, and VAL.

Generally, simultaneous quantitative analysis of multi-compound samples is difficult. One approach is applying modern analytical instruments [13–17]. According to the available literature, different analytical methods have been established for the simultaneous determination of SAC and VAL, based on reversed phase high performance liquid chromatography (RP-HPLC) method with UV detector [18–26], reversed phase ultra-performance liquid chromatography (RP-UPLC) [27], high performance thin layer chromatography (HPTLC) [28], HPLC with fluorescence detector [29], and high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [30, 31]. These methods have some drawbacks such as being time consuming, consuming large amounts of organic solvents, and requiring expensive and complicated analytical instruments. Another reported method is based on spectrofluorometric method [32], which is a not very common instrument in most laboratories.

On the other hand, simultaneous quantitative analysis of multi-compound samples by classical spectrophotometric methods is difficult or sometimes impossible due to overlapping spectra. One way for overcoming this drawback is applying chemometrics techniques such as signal processing and manipulation of the original absorption spectra.

To the best of our knowledge, only three spectrophotometric methods have been published for the simultaneous estimation of SAC and VAL based on derivative spectrophotometry [33], manipulation approaches [34], as well as bivariate and multivariate methods [35]. The main disadvantage of all these published spectrophotometric methods is the use of organic solvents for sample preparation. Also, another important disadvantage of the derivative techniques is that the signal-to-noise ratio worsens as the derivative orders grow.

The Wavelet Transform (WT) method, as a signal and image processing method in applied mathematics has attracted interest in chemical studies due to its efficiency, variety of basis functions, and fast data treatment [36]. The applications of this method for determining metals and organic compounds in different binary and multi-components matrices such as foodstuff and pharmaceutical samples [37–53] represent the potential resolution associated with them.

Accordingly, the aim of the present study was to develop a CWT method coupled with zero-crossing technique, applied on a spectrophotometric method for the
simultaneous determination of SAC and VAL in their binary mixture and combined tablet dosage form without the use of organic solvent and the time-consuming extraction step. Several optimum methods were found via this technique for plotting the calibration curve. They were then validated by analyzing several artificially prepared mixtures with known concentrations. Further, the assay results of tablets via these methods were also compared to a reference HPLC method applying one-way ANOVA test, which indicated a good agreement.

**MATERIALS AND METHODS**

*Instrumentation and software*

*Spectrophotometry*

Spectrophotometric analysis was conducted using a Shimadzu 1700 UV–visible double beam spectrophotometer equipped with two matched 1.0 cm path length quartz cells and UVProbe Ver 2.42 data handling system. For the analysis, the absorption spectra of pure standard solutions, synthetic mixtures, and real samples were recorded within the range of 190–400 nm with 1-nm intervals at room temperature against water as blank. All absorption data processing, calculations, and statistical tests were performed by Wavelet Toolbox 4.15 of MATLAB R2015b software, Microsoft Excel 2010 program, and Minitab®17 statistical software.

*High performance liquid chromatography*

The HPLC analysis (as reference method) was conducted using a Younglin (Korea) system equipped with a YL9110 quaternary pump, a YL9120 UV-Vis detector at 254 nm, a YL9101 vacuum degasser, a YL9131 column compartment, a Rhodyne 7725 manual injector with a 20 µL sample loop, and a YL-Clarity data acquisition and handling system. A 250x4.6 mm, 5-µm reverse-phase Inertsil®ODS-3V LC column (GL Science) with the related guard column was used as the stationary phase. The mobile phase was a degassed isocratic mixture of ortho-phosphoric acid (0.1% V/V in water) - acetonitrile (60:40, v/v) at a flow rate of 2 mL/min. A mixture of equal volumes of acetonitrile and water was used as diluent for stepwise preparation of standard and test solutions to reach the total SAC and VAL anions concentration of 100 µg mL⁻¹. The total HPLC analysis time was approximately 18 min with the retention times of SAC and VAL obtained as about 10.8 and 15.9 min, respectively.

*Chemicals and samples*

The working standards of SAC (purity 98.60% on anhydrous basis and calcium salt) and VAL (purity 101.0% on anhydrous basis) were kindly received from Optrix laboratories (India) as gift. ENTRESTO 100 mg tablets, as commercial pharmaceutical dosage form of SAC and VAL combination manufactured by NOVARTIS, were purchased from Turkish market. The water was supplied by an ultra-water purification system (aquaMAXTM-Ultra, Younglin, Korea). The HPLC grade acetonitrile, purchased from Duksan (South Korea) and the ortho-phosphoric acid from Merck (Darmstadt, Germany) were used for the reference method.

*Preparation of standard solutions*

The stock solutions of SAC and VAL were prepared separately in water at a concentration of 50µg mL⁻¹ by dissolving an accurately weighted amount of each compound, equivalent to 25 mg of their anhydrous anion forms in 500 mL of water. An ultrasonic bath was also used for completion of dissolution. All standard solutions were individually prepared by stepwise dilution of the stock solutions in water to reach to the concentration range between 0.5 and 25 µg mL⁻¹, after preliminary checking the validity of the Beer Lambert law.

*Preparation of synthetic binary mixtures*

A set of the various synthetic binary mixtures of two components with known concentrations within the linear ranges were prepared separately by mixing suitable volumes of each stock solutions of SAC and VAL and diluting with water. The concentrations of the synthetic binary mixtures associated with each component have been presented in Table 1.
Table 1. Concentrations of the 15 synthetic binary mixtures of SAC and VAL.

<table>
<thead>
<tr>
<th>Synthetic Mixture</th>
<th>Concentration (μg/ml)</th>
<th>SAC</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td></td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>M2</td>
<td></td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>M3</td>
<td></td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>M4</td>
<td></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>M5</td>
<td></td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>M6</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>M7</td>
<td></td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>M8</td>
<td></td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>M9</td>
<td></td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>M10</td>
<td></td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>M11</td>
<td></td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>M12</td>
<td></td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>M13</td>
<td></td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>M14</td>
<td></td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>M15</td>
<td></td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

Preparation of real samples

Ten tablets were weighed to obtain the mean weight and were then finely powdered. A portion of the tablet powder equivalent to about 50 mg of total labeled amount was accurately weighed, transferred into a 100 mL volumetric flask, and diluted to the mark with water. After magnetic stirring for about 5 min, the solution was filtered and 1 mL of the transparent solution was diluted to 50 mL with water.

Theoretical background

The philosophy of wavelet transform is based on signal processing theory. This kind of signal processing is a developed form of the Fourier transform and expressed as a set of basic functions, which decompose a signal or data into components. These functions are derived from a scaled (dilated) and shifted (translated) mother wavelet function. In other words, every generated wavelet function can be written in terms of the mother (original) wavelet transform function \( \Psi(t) \) as defined by Eq. (1):

\[
\Psi_{a,b}(t) = \frac{1}{\sqrt{|a|}} \Psi \left( \frac{t - b}{a} \right) \quad \{ a, b \in R \}
\]

Where, \( a \) is the scale (dilation) parameter and is used to change the scale of wavelet, \( b \) is the shift (translation) parameter controlling the movement of the wavelet along the time axis, and \( R \) is the domain of real numbers. Accordingly, the CWT of a signal denoted by \( f(t) \), is expressed by the Eq. (2):

\[
\text{CWT} = C(a, b) = \int_{-\infty}^{+\infty} f(t) \Psi_{a,b}^*(t)dt
\]

Where, \( \langle f(t), \Psi_{a,b} \rangle \) represents inner products or the projection of function \( f(t) \) onto the \( \Psi(t) \) function and the superscript * shows the complex conjugate for a complex wavelet [54].

Combination of wavelet transform signal with zero-crossing technique, which is based on finding the points where the signal of a mathematical function changes from positive to negative (or vice versa) provides useful signal features.
RESULTS AND DISCUSSION

**UV spectral features**

The overlay UV absorption spectra of pure SAC and VAL standard solutions are demonstrated in Figure 2. As can be observed, two components highly overlap with each other within the wavelength range of 190–400 nm. Hence, simultaneous determination of their binary mixture is not possible via classical spectrophotometric methods. Accordingly, we developed a method based on the continuous wavelet transform in combination with zero crossing-point technique.

![Figure 2. The overlay UV absorption spectra of pure SAC (-) 10μg mL⁻¹, and pure VAL (--) 10μg mL⁻¹.](image)

**Continuous wavelet transform method**

To perform continuous wavelet transform, initially the Excel file of UV absorbance data was transferred into Wavelet Toolbox domain of MATLAB software and the data were subjected to all of the various shifted mother wavelets from continuous wavelet transform family with 64 scales to find CWT coefficients ($C_{a,b}$). Then, the values of $C_{a,b}$ for each component were plotted against wavelengths to obtain CWT spectrums. In order to find the optimum CWT settings for plotting desirable calibration graphs and for valid simultaneous prediction of two components in their binary mixtures, a zero-crossing technique was applied on overlay CWT spectra of the two components. Totally, 25 optimum CWT settings were identified for SAC determination at zero-crossing points of VAL and similarly, 30 optimum CWT settings were observed for VAL at zero-crossing points of SAC.

The calibration graphs of each component were constructed by plotting the CWT signal amplitude in each setting (corresponding to the zero-crossing points of the other) against the related concentration. The obtained linear regression equations were used for predicting the concentrations of one component in its binary mixtures with the other. These optimum settings and their corresponding statistical results of linear regression are summarized in Tables 2 and 3. As can be seen in these tables, all the statistical parameters of linear regressions lied within the acceptable range for the quantification of each component in the presence of the other.
Table 2. Statistical results of linear regression of SAC at zero-cross point of VAL.

<table>
<thead>
<tr>
<th>Mother Wavelet</th>
<th>Scale</th>
<th>$\lambda$ (nm)</th>
<th>LR $^*$ (μg mL$^{-1}$)</th>
<th>Regression Equation</th>
<th>$R^2$</th>
<th>S $^*$</th>
<th>LOD $^*$ (μg mL$^{-1}$)</th>
<th>LOQ $^*$ (μg mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bior2.2</td>
<td>47</td>
<td>221</td>
<td>1.00-16.00</td>
<td>$Y = -0.215 X - 0.0331$</td>
<td>0.9996</td>
<td>0.0183</td>
<td>0.26</td>
<td>0.85</td>
</tr>
<tr>
<td>bior2.2</td>
<td>58</td>
<td>266</td>
<td>1.00-25.00</td>
<td>$Y = 0.1253 X + 0.0164$</td>
<td>0.9999</td>
<td>0.0113</td>
<td>0.27</td>
<td>0.90</td>
</tr>
<tr>
<td>coif3</td>
<td>63</td>
<td>231</td>
<td>1.00-16.00</td>
<td>$Y = -0.1552 X - 0.0249$</td>
<td>0.9997</td>
<td>0.0126</td>
<td>0.24</td>
<td>0.81</td>
</tr>
<tr>
<td>coif3</td>
<td>46</td>
<td>265</td>
<td>2.00-25.00</td>
<td>$Y = 0.0996 X + 0.0293$</td>
<td>0.9995</td>
<td>0.0168</td>
<td>0.51</td>
<td>1.69</td>
</tr>
<tr>
<td>db5</td>
<td>36</td>
<td>239</td>
<td>2.00-20.00</td>
<td>$Y = 0.128 X + 0.0416$</td>
<td>0.9990</td>
<td>0.0223</td>
<td>0.52</td>
<td>1.74</td>
</tr>
<tr>
<td>db5</td>
<td>37</td>
<td>278</td>
<td>2.00-22.00</td>
<td>$Y = -0.0884 X - 0.022$</td>
<td>0.9995</td>
<td>0.0120</td>
<td>0.41</td>
<td>1.36</td>
</tr>
<tr>
<td>dmey</td>
<td>61</td>
<td>232</td>
<td>1.00-16.00</td>
<td>$Y = -0.1582 X - 0.024$</td>
<td>0.9997</td>
<td>0.0119</td>
<td>0.23</td>
<td>0.75</td>
</tr>
<tr>
<td>dmey</td>
<td>46</td>
<td>258</td>
<td>2.00-22.00</td>
<td>$Y = 0.1497 X + 0.0467$</td>
<td>0.9993</td>
<td>0.0254</td>
<td>0.51</td>
<td>1.70</td>
</tr>
<tr>
<td>flk8</td>
<td>62</td>
<td>233</td>
<td>1.00-16.00</td>
<td>$Y = -0.1408 X - 0.022$</td>
<td>0.9997</td>
<td>0.0112</td>
<td>0.24</td>
<td>0.79</td>
</tr>
<tr>
<td>flk8</td>
<td>33</td>
<td>249</td>
<td>1.00-16.00</td>
<td>$Y = 0.0964 X + 0.0094$</td>
<td>0.9999</td>
<td>0.0048</td>
<td>0.15</td>
<td>0.49</td>
</tr>
<tr>
<td>gaus1</td>
<td>13</td>
<td>206</td>
<td>0.50-12.00</td>
<td>$Y = 0.1209 X + 0.0135$</td>
<td>0.9999</td>
<td>0.0059</td>
<td>0.15</td>
<td>0.49</td>
</tr>
<tr>
<td>haar</td>
<td>58</td>
<td>209</td>
<td>2.00-16.00</td>
<td>$Y = 0.136 X + 0.0404$</td>
<td>0.9990</td>
<td>0.0180</td>
<td>0.16</td>
<td>0.33</td>
</tr>
<tr>
<td>meyr</td>
<td>10</td>
<td>254</td>
<td>1.00-25.00</td>
<td>$Y = 0.0865 X + 0.0058$</td>
<td>0.9999</td>
<td>0.0048</td>
<td>0.17</td>
<td>0.55</td>
</tr>
<tr>
<td>meyr</td>
<td>8</td>
<td>217</td>
<td>0.50-12.00</td>
<td>$Y = -0.0718 X - 0.0051$</td>
<td>0.9999</td>
<td>0.0035</td>
<td>0.15</td>
<td>0.49</td>
</tr>
<tr>
<td>meyr</td>
<td>55</td>
<td>215</td>
<td>2.00-16.00</td>
<td>$Y = 0.1173 X + 0.0304$</td>
<td>0.9993</td>
<td>0.0129</td>
<td>0.33</td>
<td>1.10</td>
</tr>
<tr>
<td>meyr</td>
<td>50</td>
<td>284</td>
<td>2.00-22.00</td>
<td>$Y = 0.1536 X + 0.0471$</td>
<td>0.9993</td>
<td>0.0258</td>
<td>0.50</td>
<td>1.68</td>
</tr>
<tr>
<td>morl</td>
<td>47</td>
<td>221</td>
<td>1.00-16.00</td>
<td>$Y = -0.1344 X - 0.0212$</td>
<td>0.9997</td>
<td>0.0113</td>
<td>0.25</td>
<td>0.84</td>
</tr>
<tr>
<td>morl</td>
<td>52</td>
<td>257</td>
<td>2.00-20.00</td>
<td>$Y = 0.145 X + 0.0374$</td>
<td>0.9993</td>
<td>0.0208</td>
<td>0.43</td>
<td>1.43</td>
</tr>
<tr>
<td>morl</td>
<td>53</td>
<td>291</td>
<td>2.00-22.00</td>
<td>$Y = -0.0966 X - 0.023$</td>
<td>0.9996</td>
<td>0.0129</td>
<td>0.4</td>
<td>1.34</td>
</tr>
<tr>
<td>morl</td>
<td>55</td>
<td>327</td>
<td>1.00-22.00</td>
<td>$Y = 0.0477 X + 0.0043$</td>
<td>0.9998</td>
<td>0.0043</td>
<td>0.27</td>
<td>0.90</td>
</tr>
<tr>
<td>rbio3.3</td>
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<td>209</td>
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<td>$Y = 0.1263 X + 0.0364$</td>
<td>0.9991</td>
<td>0.0158</td>
<td>0.38</td>
<td>1.25</td>
</tr>
<tr>
<td>rbio3.3</td>
<td>27</td>
<td>241</td>
<td>2.00-22.00</td>
<td>$Y = 0.1375 X - 0.0378$</td>
<td>0.9994</td>
<td>0.0207</td>
<td>0.45</td>
<td>1.50</td>
</tr>
<tr>
<td>sym5</td>
<td>48</td>
<td>232</td>
<td>2.00-20.00</td>
<td>$Y = 0.1944 X + 0.0589$</td>
<td>0.9991</td>
<td>0.0322</td>
<td>0.50</td>
<td>1.65</td>
</tr>
<tr>
<td>sym5</td>
<td>55</td>
<td>274</td>
<td>1.00-25.00</td>
<td>$Y = -0.1039 X - 0.011$</td>
<td>0.9999</td>
<td>0.0073</td>
<td>0.21</td>
<td>0.70</td>
</tr>
</tbody>
</table>

$^*$ Linear Range; $^\dagger$ Coefficient of Determination; $^\ddagger$ Standard Error of the Regression; $^\S$ Limit of Detection; $^\D$ Limit of Quantification

Table 3. Statistical results of linear regression of VAL at zero-cross point of SAC.

<table>
<thead>
<tr>
<th>Mother Wavelet</th>
<th>Scale</th>
<th>$\lambda$ (nm)</th>
<th>LR $^*$ (μg mL$^{-1}$)</th>
<th>Regression Equation</th>
<th>$R^2$</th>
<th>S $^*$</th>
<th>LOD $^*$ (μg mL$^{-1}$)</th>
<th>LOQ $^*$ (μg mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bior2.2</td>
<td>50</td>
<td>242</td>
<td>2.00-25.00</td>
<td>$Y = -0.0532 X - 0.0074$</td>
<td>0.9994</td>
<td>0.0090</td>
<td>0.51</td>
<td>1.69</td>
</tr>
<tr>
<td>coif3</td>
<td>57</td>
<td>218</td>
<td>1.00-25.00</td>
<td>$Y = 0.158 X - 0.0274$</td>
<td>0.9999</td>
<td>0.0124</td>
<td>0.23</td>
<td>0.78</td>
</tr>
<tr>
<td>coif3</td>
<td>54</td>
<td>248</td>
<td>2.00-20.00</td>
<td>$Y = -0.0933 X + 0.0031$</td>
<td>0.9991</td>
<td>0.0155</td>
<td>0.50</td>
<td>1.66</td>
</tr>
<tr>
<td>db5</td>
<td>58</td>
<td>203</td>
<td>1.00-20.00</td>
<td>$Y = 0.121 X - 0.0217$</td>
<td>0.9999</td>
<td>0.0078</td>
<td>0.19</td>
<td>0.65</td>
</tr>
<tr>
<td>db5</td>
<td>53</td>
<td>237</td>
<td>1.00-20.00</td>
<td>$Y = -0.1447 X + 0.0135$</td>
<td>0.9997</td>
<td>0.0138</td>
<td>0.29</td>
<td>0.95</td>
</tr>
<tr>
<td>db5</td>
<td>63</td>
<td>294</td>
<td>2.00-20.00</td>
<td>$Y = 0.0826 X + 0.0011$</td>
<td>0.9993</td>
<td>0.0120</td>
<td>0.43</td>
<td>1.45</td>
</tr>
<tr>
<td>dmey</td>
<td>54</td>
<td>220</td>
<td>1.00-25.00</td>
<td>$Y = 0.1357 X - 0.0219$</td>
<td>0.9999</td>
<td>0.0095</td>
<td>0.21</td>
<td>0.70</td>
</tr>
<tr>
<td>dmey</td>
<td>63</td>
<td>257</td>
<td>2.00-20.00</td>
<td>$Y = -0.1298 X + 0.0114$</td>
<td>0.9994</td>
<td>0.0183</td>
<td>0.42</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Table 3. Continued.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample Size</th>
<th>Limit of Detection</th>
<th>Limit of Quantification</th>
<th>RSD%</th>
<th>RSD%</th>
<th>Recovery %</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>rBio3.3</td>
<td>62</td>
<td>207</td>
<td>0.50-12.00</td>
<td>0.0723 X + 0.0069</td>
<td>1.0723</td>
<td>0.0016</td>
<td>0.0723</td>
</tr>
<tr>
<td>rBio3.3</td>
<td>52</td>
<td>241</td>
<td>1.00-20.00</td>
<td>0.1714 X + 0.0125</td>
<td>0.1714</td>
<td>0.0161</td>
<td>0.1714</td>
</tr>
<tr>
<td>rBio3.3</td>
<td>52</td>
<td>252</td>
<td>0.50-20.00</td>
<td>0.1165 X - 0.0128</td>
<td>0.1165</td>
<td>0.0045</td>
<td>0.1165</td>
</tr>
<tr>
<td>sym5</td>
<td>59</td>
<td>226</td>
<td>1.00-25.00</td>
<td>-0.1625 X + 0.0152</td>
<td>-0.1625</td>
<td>0.0125</td>
<td>-0.1625</td>
</tr>
<tr>
<td>sym5</td>
<td>60</td>
<td>255</td>
<td>1.00-10.00</td>
<td>0.0529 X - 0.01</td>
<td>0.0529</td>
<td>0.0052</td>
<td>0.0529</td>
</tr>
</tbody>
</table>

*Linear Range; Coefficient of Determination; Standard Error of the Regression; Limit of Detection; Limit of Quantification

**Analysis of synthetic binary mixtures and selection of the best methods**

To assess the prediction ability of the proposed CWT methods for the simultaneous determination of SAC and VAL and to choose the best methods, 15 various synthetic binary mixtures were tested and mean %recovery values along with the relative standard deviation were calculated. Then, an ANOVA test at 95% confidence level for all two-sided confidence intervals was performed to evaluate the obtained results. The low p-value (0.000) indicated that there is sufficient evidence that not all the means are equal when alpha is set at 0.05, suggesting a significant difference between mean %recovery values of the proposed methods for both SAC and VAL. To explore the differences among the means, Fisher pairwise comparisons were applied and the proposed methods were grouped. Then, the proposed methods with the best mean %recovery and %RSD values and the maximum number of groups were selected. Table 4 presents the mean %recovery values of SAC and VAL along with the relative standard deviation of the selected proposed methods for SAC and VAL.
Table 4. %Recovery data obtained applying the proposed methods to synthetic binary mixtures.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mother Wavelet</th>
<th>Scale</th>
<th>λ (nm)</th>
<th>% Recovery*</th>
<th>% RSDb</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAC</td>
<td>dmey</td>
<td>61</td>
<td>232</td>
<td>99.10</td>
<td>1.06</td>
</tr>
<tr>
<td>SAC</td>
<td>sym5</td>
<td>48</td>
<td>232</td>
<td>99.09</td>
<td>0.94</td>
</tr>
<tr>
<td>VAL</td>
<td>db5</td>
<td>53</td>
<td>237</td>
<td>101.10</td>
<td>0.79</td>
</tr>
<tr>
<td>VAL</td>
<td>meyr</td>
<td>50</td>
<td>272</td>
<td>101.16</td>
<td>0.82</td>
</tr>
<tr>
<td>VAL</td>
<td>meyr</td>
<td>59</td>
<td>247</td>
<td>100.19</td>
<td>0.67</td>
</tr>
<tr>
<td>VAL</td>
<td>sym5</td>
<td>59</td>
<td>226</td>
<td>100.33</td>
<td>1.23</td>
</tr>
</tbody>
</table>

* Mean value; b Relative standard deviation

Validation of the proposed methods

The analytical performance of the proposed methods, obtained by method validation process, is presented in Tables 2, 3, and 4. The high values of coefficient of determination ($R^2$ larger than 0.999) suggest a good linear relationship between CWT signal value and concentration for both drugs. The LOD and LOQ, reported in Tables 2 and 3, were defined as 3 S/m and 10 S/m, where S is the standard error of the regression and m is the slope of calibration curve. The excellent %recovery values (99.09–101.16%) indicate the high accuracy of the proposed methods. Further, the %RSD (Max. 1.23%) suggests the high precision of the methods. Also, the results of applying the proposed methods on placebo (excipients) showed no interferences, proving the specificity of them. The obtained results confirm the validity of the proposed methods for simultaneous determination of SAC and VAL. The CWT signals for calibration sets of SAC and VAL obtained by plotting $C_{a,b}$ coefficients versus wavelengths via the proposed methods are depicted in Figures 3 and 4.

![Figure 3](image-url)

**Figure 3.** CWT signals for calibration sets of SAC: (a) dmey (scale=61, $\lambda$= 232nm) CWT, and (b) sym5 (scale=48, $\lambda$= 232nm) CWT.
Figure 4. CWT signals for calibration sets of VAL: (a) db5 (scale=53, λ= 237nm) CWT, (b) meyr (scale=50, λ= 272nm) CWT, (c) meyr (scale=59, λ= 247nm) CWT, and (d) sym5 (scale=59, λ= 226nm) CWT.
Analysis of real samples

To evaluate the effect of the real sample matrix of the proposed method, ENTRESTO 100 mg tablets, as commercial pharmaceutical dosage form, were used. Also, the proposed methods were applied to simultaneous quantitative determination of SAC and VAL, with its results summarized in Table 5. One-way ANOVA test at 95% confidence level for all two-sided confidence intervals was performed in order to find possible significant differences between the results of each proposed method, with the results obtained by the reference method. Table 6 outlines the results of ANOVA test. Since the p-values (0.583, 0.298) were far larger than 0.05, it can be concluded that at 95% confidence level there are no significant differences between the results of the proposed methods and reference method. So, the accuracy and precision of the proposed methods is sufficient in order to be used in routine quality control of pharmaceutical dosage forms.

Table 5. %Recovery data obtained applying the proposed methods and reference method to real samples (ENTRESTO).

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte</th>
<th>Labeled Amount (mg per tablet)</th>
<th>% Recovery a</th>
<th>% RSD b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dmey (scale=61, λ= 232nm)</td>
<td>SAC</td>
<td>49</td>
<td>102.16 ± 1.22</td>
<td>1.19</td>
</tr>
<tr>
<td>sym5(scale=48, λ= 232nm)</td>
<td>SAC</td>
<td>49</td>
<td>102.82 ± 0.591</td>
<td>0.58</td>
</tr>
<tr>
<td>HPLC</td>
<td>SAC</td>
<td>49</td>
<td>101.86 ± 1.35</td>
<td>1.32</td>
</tr>
<tr>
<td>db5(scale=53, λ= 237nm)</td>
<td>VAL</td>
<td>51</td>
<td>101.73 ± 1.10</td>
<td>1.08</td>
</tr>
<tr>
<td>Meyr (scale=50, λ= 272nm)</td>
<td>VAL</td>
<td>51</td>
<td>100.68 ± 0.685</td>
<td>0.68</td>
</tr>
<tr>
<td>Meyr (scale=59, λ= 247nm)</td>
<td>VAL</td>
<td>51</td>
<td>100.40 ± 0.436</td>
<td>0.43</td>
</tr>
<tr>
<td>sym5(scale=59, λ= 226nm)</td>
<td>VAL</td>
<td>51</td>
<td>101.99 ± 1.01</td>
<td>0.99</td>
</tr>
<tr>
<td>HPLC</td>
<td>VAL</td>
<td>51</td>
<td>100.98 ± 1.43</td>
<td>1.42</td>
</tr>
</tbody>
</table>

a Mean of three determination; b Relative standard deviation

Table 6. The ANOVA test results by applying the proposed and reference methods to the real samples at a confidence level of 95%.

<table>
<thead>
<tr>
<th>Source of variations</th>
<th>DF a</th>
<th>Adj. SS b</th>
<th>Adj. MS c</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor (method)</td>
<td>2</td>
<td>1.444</td>
<td>0.7219</td>
<td>0.59</td>
<td>0.583</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>7.337</td>
<td>1.2229</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8.781</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variations</th>
<th>DF</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor(method)</td>
<td>4</td>
<td>5.589</td>
<td>1.3973</td>
<td>1.41</td>
<td>0.298</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>9.882</td>
<td>0.9882</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>15.471</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Degree of freedom; b Adjusted sum of squares; c Adjusted mean squares

CONCLUSIONS

In this work, a rapid, accurate, precise, and environmentally friendly spectrophotometric method coupled with CWT-zero crossing technique was introduced for simultaneous determination of SAC and VAL in tablet dosage form.
containing their newly FDA approved drug. Validation of the 25 and 30 identified settings for SAC and VAL determination at zero-crossing points of each other respectively was performed by artificial synthetic binary mixtures. Furthermore, optimum CWT families were selected applying ANOVA test and Fisher pairwise comparisons. No requirement of using organic solvent has been the main advantage of the proposed methods compared to other published spectrophotometric methods. Further, the proposed methods have reasonably good analytical performance including high recovery, low LOD, low LOQ, high precision, $R^2$ larger than 0.999, rapidity, and low cost. Considering all the above, it is more economic to replace expensive and time-consuming HPLC method by this method in quality control laboratories.

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