Simultaneous spectrophotometric determination of triamterene and hydrochlorothiazide in Triamterene-H tablets using continuous wavelet transformation

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Abstract: A spectrophotometric method for simultaneous analysis of triamterene and hydrochlorothiazide is proposed by application of continuous wavelet transform on the spectral data without time-consuming extraction step. Different wavelets families were tested and Coiflets wavelet family (coif1) and Reverse biorthogonal wavelets family (rbio 2.8) were selected and applied under the optimal conditions for simultaneous analysis.

The validation of the developed methods was confirmed by analyzing various synthetic mixtures of the investigated drugs. Triamterene and hydrochlorothiazide could be determined simultaneously in the range of 0.75-5.0 µg ml⁻¹ and 1.0–6.0 µg.ml⁻¹ respectively and LOD for triamterene and hydrochlorothiazide was less than 1.07 µg.ml⁻¹ and 0.89 µg.ml⁻¹ respectively.

The experimental results obtained from the continuous wavelet transform approach were statistically compared with those obtained by derivative spectrophotometry and successful results were reported.

Keywords: Continuous wavelet transform; Simultaneous determination; triamterene; hydrochlorothiazide

Introduction

Triamterene(2,4,7-tri-amino-6-phenylpteridine) (TRM) is used commonly in combination with hydrochlorothiazide (6chloro-3,4-dihydro-2H-1, 2,4 benzothiadiazine-7-sulphonamide1,1-dioxide) (HYD). The mixture of these two drugs is used in treatment for reducing edema and medium hypertension. This combination, Triamterene-H, is commercialized for human treatment. Therefore, the determination of these drugs is a frequent analytical problem in quality control of the pharmaceutical industries. The two drugs studied in this work show a strong overlap between their absorption spectra. Hence, their simultaneous determination is hard when conventional spectrophotometric techniques are used [1–3]. Normally, the method used to resolve a complex mixture of these drugs is mainly high-performance liquid chromatography (HPLC) [4,5]. These methods are time consuming and require complicated and expensive instruments.
A wavelet transform (WT) is the representation of a function by wavelets. The wavelets are scaled and translated copies of a finite-length or fast-decaying oscillating waveform. WT to overcome the problem of noisy and incomplete data has been successfully put on a sound statistical basis by Donoho and co-workers [6, 7]. WT was applied to denoising of data [8, 9], compressing of signals [10, 11], simultaneous determination of chemical species in binary [12-24], and ternary [25-27] mixtures and determination of enantiomeric ratio [11]. In this study, we developed a continuous wavelet transform (CWT) method for the simultaneous analysis of triamterene and hydrochlorothiazide in Triamterene-H tablets, with no separation steps. The experimental results obtained were statistically compared with those obtained by derivative spectrophotometry (DS) and successful results were reported. The method was validated by simultaneous determination of triamterene and hydrochlorothiazide in some synthetic samples.

Methodology

Wavelet method

Wavelet transform is a novel signal processing technique developed from the Fourier transform. Wavelet theory is based on analyzing signals to their components by using a set of basis functions. The analyzing function of wavelet transform is the wavelet, which is a family of functions derived from a basic function, called mother wavelet by dilation (or scaling) and translation.

Let us choose a mother wavelet [28, 29] by \( \Psi (\lambda) \). By using scaling and shifting on it, a set of functions denoted by \( \Psi_{a,b}(t) \) is obtained as follows:

\[
\Psi_{a,b}(t) = \frac{1}{\sqrt{a}} \Psi \left( \frac{t-b}{a} \right) \quad \text{for} \quad a, b \in \mathbb{R}, \quad a \neq 0
\]  

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.

CWT of a given signal denoted by \( f(t) \) is defined by the following formula:

\[
C(a, b) = \int_{-\infty}^{\infty} f(t) \overline{\Psi_{a,b}(t)} dt \quad \text{<} f(t) \overline{\Psi_{a,b}} >
\]

Where the superscript * represents the complex conjugate and \( <f(t), \Psi_{a,b}> \) denotes the inner product of function \( f(t) \) onto the wavelet function \( \Psi_{a,b}(t) \).

The wavelet \( \Psi \) is called invertible if it fulfills the following condition

\[
Coa = \int_{-\infty}^{\infty} |\Psi(\omega)|^2 d\omega < \infty
\]

Experimental

Apparatus and software

A Bio-TEK kon922 double beam UV–vis spectrophotometer with a 1-cm path length quartz cell was used. Calculations and the signal transforms were obtained by using EXCEL and MATLAB 7.1.

Chemicals

Pharmaceutical grade triamterene and Pharmaceutical grade hydrochlorothiazide were used and analytical reagent-grade solvent was purchased from Merck (Darmstadt, Germany).

Standard Solutions

Stock standard solutions of triamterene and hydrochlorothiazide were prepared by dissolving 10.0 mg of triamterene and 10.0 mg of hydrochlorothiazide in methanol in 100 mL volumetric flasks and diluted to the mark with the solvent. Working solutions were prepared by appropriate dilution of the stock solution in methanol to reach concentration ranges of 0.75-5 and 1-6 µg ml\(^{-1}\) for triamterene and hydrochlorothiazide, respectively.

Preparation of real samples

Ten tablets were finely powdered and an appropri-
ate portion (equivalent to the median mass of one tablet) was dissolved in 100 ml of methanol. It was mechanically shaken for a period of 20 min and filtered into a 250 ml calibrated flask. The residue was washed twice with the same solvent and diluted to the volume. Into a 50 ml volumetric flask, 1 ml of this solution was transferred. The solution was then completed to the volume with methanol. A solution containing 4 µg ml\(^{-1}\) TRM and 2 µg ml\(^{-1}\) HYD was obtained.

**Results and discussion**

**UV–Vis spectra**

Figure 1 shows the spectra of TRM and HYD overlap with each other, and therefore each compound interfere in the spectrophotometric determination of the other. Classical methods can not resolve this system. But these systems can be suitable for simultaneous determination using continuous wavelet and derivative transforms of the absorption spectra in combination with zero-crossing technique.

Comparison of the spectrum of an artificial binary mixture and that of a real sample of similar concentration showed that excipients placed in the commercial preparation did not interfere the determination of TRM and HYD. (Figure 2)

**Continuous Wavelet transform method**

Various wavelet families with different scales were tested to find the optimal signal processing for obtaining desirable calibration graphs and reliable determination of triamterene and hydrochlorothiazide. Coiflets wavelet family (coif1) with \(a = 40\) and Reverse birothogonal wavelet family (rbio2.8) with \(a = 40\) were selected as optimal ones, because they gave the highest sensitivity with big slope values in the linear regression equations for all subjected compounds.

The absorption spectra of the standard solutions of TRM and hydrochlorothiazide were transferred into wavelet domain by coif1 and rbio2.8 and CWT spectra were obtained by plotting \(C_{a,b}\) coefficients versus wavelengths as could be seen in Figures 3 and 4.
The calibration graphs of TRM for the wavelet methods were constructed by plotting the transformed signals versus the concentration at the zero-crossing points of HYD and vice versa. The calibration graphs of coif1 were obtained by measuring the transformed signals at 234 and 375 nm for TRM (corresponding to zero crossing point of HYD) and at 247 and 269 nm for HYD (corresponding to zero crossing point of TRM). By using a similar procedure, the calibration graphs for rbio2.8 were constructed by measuring the signal amplitude at 233 and 260 nm for TRM and at 245 and 275 nm for HYD. Their statistical results were summarized in Table 1 The correlation coefficients of equations were calculated as higher than 0.99 and also other statistical parameters are in acceptable range for the utilization of calibration lines for the determination of both drugs.

Table 1 Linear regression analysis and its statistical results

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte</th>
<th>Linear range (µg ml⁻¹)</th>
<th>Slope (a)*</th>
<th>Intercept (b)*</th>
<th>Corr. Coeff. (r)*</th>
<th>LOD** (µg ml⁻¹)</th>
<th>LOQ*** (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coif λ=247</td>
<td>HYD</td>
<td>1-6</td>
<td>-0.1727</td>
<td>-0.0259</td>
<td>0.9994</td>
<td>0.24</td>
<td>0.79</td>
</tr>
<tr>
<td>Coif λ=269</td>
<td>HYD</td>
<td>1-6</td>
<td>0.1447</td>
<td>-0.0040</td>
<td>0.9999</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Coif λ=234</td>
<td>TRM</td>
<td>0.75-5</td>
<td>0.2185</td>
<td>0.1605</td>
<td>0.9988</td>
<td>0.29</td>
<td>0.96</td>
</tr>
<tr>
<td>Coif λ=375</td>
<td>TRM</td>
<td>0.75-5</td>
<td>0.1027</td>
<td>0.0653</td>
<td>0.9993</td>
<td>0.22</td>
<td>0.75</td>
</tr>
<tr>
<td>Rbio λ=245</td>
<td>HYD</td>
<td>1-6</td>
<td>-0.1641</td>
<td>-0.0291</td>
<td>0.9992</td>
<td>0.27</td>
<td>0.89</td>
</tr>
<tr>
<td>Rbio λ=275</td>
<td>HYD</td>
<td>1-6</td>
<td>0.1028</td>
<td>0.0039</td>
<td>0.9998</td>
<td>0.15</td>
<td>0.49</td>
</tr>
<tr>
<td>Rbio λ=233</td>
<td>TRM</td>
<td>0.75-5</td>
<td>0.1558</td>
<td>0.1161</td>
<td>0.9988</td>
<td>0.29</td>
<td>0.98</td>
</tr>
<tr>
<td>Rbio λ=260</td>
<td>TRM</td>
<td>0.75-5</td>
<td>-0.1009</td>
<td>-0.0879</td>
<td>0.9985</td>
<td>0.32</td>
<td>1.07</td>
</tr>
<tr>
<td>DS λ=243</td>
<td>TRM</td>
<td>0.75-5</td>
<td>-5.2×10⁻³</td>
<td>-4.1×10⁻³</td>
<td>0.9990</td>
<td>0.26</td>
<td>0.88</td>
</tr>
<tr>
<td>DS λ=233</td>
<td>HYD</td>
<td>1-6</td>
<td>-7.6×10⁻³</td>
<td>-1.2×10⁻³</td>
<td>0.9995</td>
<td>0.22</td>
<td>0.72</td>
</tr>
</tbody>
</table>

*a, b and r are slope, intercept and correlation coefficient of regression function, respectively.
**LOD, limit of detection
***LOQ, limit of quantification
Derivative spectrophotometry

In this method, the calibration graphs of TRM and HYD were obtained by measuring the \( \frac{dA}{d\lambda} \) values at 243 and 233 nm corresponding to zero-crossing points, respectively. The zero crossing points give the opportunity of determining TRM and HYD amount in their mixture without any priori separation step. The derivative spectra of the standard solutions of TRM and HYD are shown in Figure 5.

Table 2 shows the statistical parameters corresponding to the calibration graphs for both drugs in the chosen wavelengths.

In the optimization of the experimental conditions of DS, the effect of \( \Delta \lambda \) was tested to find the best derivative spectra. The value of \( \Delta \lambda = 10 \text{ nm} \) in the first derivative of the absorption spectra were found as considerable value for both drugs.

![First derivative spectra of HYD and TRM](image)

**Figure 5 First derivative spectra of 1, 1.5, 2, 3, 5 and 6 µg ml\(^{-1}\) HYD (—) and 0.75, 0.96, 1.6, 3.5, 4 and 5 µg ml\(^{-1}\) TRM (---).**

### Table 2 Recovery results obtained from the binary mixtures by the proposed analytical approaches

<table>
<thead>
<tr>
<th>Mixture (µg ml(^{-1}))</th>
<th>TRM Recovery (%)</th>
<th>HYD Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CWT (coif)</td>
<td>CWT (rbio)</td>
</tr>
<tr>
<td>TRM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>99.1</td>
<td>99.6</td>
</tr>
<tr>
<td>2</td>
<td>99.5</td>
<td>100.1</td>
</tr>
<tr>
<td>4</td>
<td>100.3</td>
<td>99.1</td>
</tr>
<tr>
<td>5</td>
<td>98.4</td>
<td>98.6</td>
</tr>
<tr>
<td>2</td>
<td>99.5</td>
<td>100.5</td>
</tr>
<tr>
<td>2</td>
<td>98.2</td>
<td>99.4</td>
</tr>
<tr>
<td>2</td>
<td>97.5</td>
<td>97.4</td>
</tr>
<tr>
<td>2</td>
<td>98.4</td>
<td>98.2</td>
</tr>
<tr>
<td>Mean</td>
<td>98.9</td>
<td>99.1</td>
</tr>
<tr>
<td>R.S.D.*</td>
<td>0.91</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Method validation

The validity of the proposed method was tested by using various mixtures containing TRM and HYD. Mean recoveries and the relative standard deviations were calculated and their results were given in Table 2. The accuracy and reproducibility is evident from the data as mean recoveries are close to 100% and low relative standard deviation.

Analysis of real sample

A commercial Triamterene-H tablet produced by the Irandaru factory was analyzed using continuous wavelet transform and derivative spectrophotometry method. Results are summarized in Table 3.
The results obtained by the proposed methods were compared with each other using one-way ANOVA test. The calculated F-values ($P = 0.05$) were less than the tabulated F-values. Therefore, there are not significant errors in the simultaneous determination of TRM and HYD by proposed methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>TRM (mg per tablet)</th>
<th>HYD (mg per tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$ (m)</td>
<td>CWT (coif)</td>
<td>CWT (rbio)</td>
</tr>
<tr>
<td>Mean</td>
<td>234</td>
<td>375</td>
</tr>
<tr>
<td>R.S.D.</td>
<td>50.38</td>
<td>50.13</td>
</tr>
</tbody>
</table>

**Conclusion**

A spectrophotometric method by application of continuous wavelet transform was developed to analyze trimetere and hydrochlorothiazide simultaneously in Triamterene-H tablets. The results obtained were compared with those obtained by derivative spectrophotometry method and successful results were reported. The proposed methods are rapid, simple, sensitive, precise, without time-consuming extraction step and very cheap for routine analysis.

CWT method has many advantages such as high amplitude of wavelet transform graphs to improve the sensitivity, many zero crossing points to eliminate probable interferences or to find the best points, many wavelet families to find the optimum one and diminishing of the noise.

Good results were obtained by utilizing CWT showed that proposed method is appropriate for the quality control and the routine analysis in the mixtures and commercial products.

**References**

Simultaneous spectrophotometric determination...