Simultaneous Spectrophotometric Determination of Tramadol and Acetaminophen in Pharmaceutical Formulations Using H-Point Standard Addition Method

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ABSTRACT

H-point standard addition method (HPSAM) was applied to the simultaneous spectrophotometric determination of tramadol and acetaminophen in pharmaceutical formulations. The results showed that tramadol and acetaminophen can be determined simultaneously in the range 5.0-100 and 1.0-10 µg/ml, respectively. The limits of detection (LOD) for tramadol and acetaminophen were obtained 2.0 and 0.50 µg/ml, respectively. Relative standard deviation for five replicate simultaneous determinations of tramadol and acetaminophen were calculated to be 3.2 and 3.3%, respectively. The proposed method has been successfully applied to the simultaneous determination of tramadol and acetaminophen in some synthetic, pharmaceutical formulation.

Keywords: Tramadol, Acetaminophen, Spectrophotometric, H-point standard addition method, Pharmaceutical formulation.

INTRODUCTION

Tramadol is a synthetic monoamine uptake inhibitor and centrally acting analgesic, used for treating moderate to severe pain and it appears to have actions at the µ-opioid receptor as well as the noradrenergic and serotonergic systems. Acetaminophen or paracetamol is a widely used analgesic and antipyretic drug. It is well tolerated, lacks many of the side effects of aspirin, so it is commonly used for the relief of fever, headaches, and other minor aches, pains and the management of more severe pain, where it allows lower dosages of additional non-steroidal anti-inflammatory drugs to be used, thereby minimizing overall side effects. In relation to the effectiveness in pharmaceutical formulations, the mechanisms of action of two analgesics (37.5 mg tramadol and 325 mg acetaminophen), combining these drugs as a fixed tablet, enhance the analgesic effectiveness, reduce the side effects and also increase the drug’s compliance and provide effective analgesia in patients with moderate to severe acute pain and those with chronic painful conditions characterized by intermittent exacerbations of pain. The importance of this pharmaceutical combination has been demonstrated by the World Health Organization (WHO) analgesic scale in level II pain treatment. Therefore, it is necessary to develop a rapid, precise, simple and reliable method for the determination of tramadol and acetaminophen in pharmaceutical preparations.

In spite of publishing several reports about the individual determination of tramadol and acetaminophen, only a few reports have been published on the simultaneous determination of two analytes in pharmaceutical formulations, which include liquid chromatography, electrochemical, and spectrophotometry.
Among the various procedures for quantitation of chemical species, spectrophotometric methods play a prominent role, but the selectivity of most spectrophotometric procedures are not satisfactory, owing to the close related properties, and separation procedures that must be introduced in most cases. For achieving quantitative information from such systems without previous chemical separation, several chemometric methods have been developed. Some of these procedures are based on the use of multivariate data, such as regression on latent variables (principal component regression, PCR and partial least squares, PLS) and other methods were presented which use bivariate data such as H-point standard addition method (HPSAM).

Bosch-Reig and CampinsFalco presented a technique called the H-point standard addition method that was based on the principle of dual-wavelength spectrophotometry and the standard addition method.16 The greatest advantage of the HPSAM is that it can remove the presence of an interference and reagent blank. The HPSAM can determine the two components simultaneously with extensively or even coincident overlapping spectra.17 So far, several reports have been published on the simultaneous determination of drugs in pharmaceutical formulations by using spectrophotometric H-point standard addition method (SHPSAM).18-24

The main goal of the present report is developing a simple, selective and accurate method for the simultaneous determination of tramadol and acetaminophen by using H-point standard addition method. This method has been successfully applied for simultaneous determination of tramadol and acetaminophen in synthetic samples.

MATERIALS AND METHODS

Apparatus
A Hitachi model 3310 UV-V spectrophotometer with 1-cm quartz cells was used for recording the absorbance spectra. All spectral measurements were performed by using a blank solution as a reference.

Chemicals
Pharmaceutical grade tramadol and acetaminophen were obtained as a gift sample from local pharmaceutical companies. Tablets of tramadol hydrochloride, acetaminophen, and combined dosage forms were purchased from the local market for analysis. Stock solutions of tramadol and acetaminophen (1000 µg/ml) were prepared by dissolving appropriate volumes of each drug in methanol and diluting to the mark in a 100 ml volumetric flask. Working solutions were prepared by diluting the standard solution in water.

Recommended Procedure
Appropriate volumes of tramadol and acetaminophen were added into a 10 ml volumetric flask. The solution was diluted to the mark. A portion of the solution was transferred into a 1 cm quartz cell to measure the absorbance at appropriate wavelengths. Synthetic samples containing different concentrations of tramadol and acetaminophen standard solutions were prepared, and a standard addition of acetaminophen (up to 6.0 µg/ml) was made. The simultaneous determination of tramadol and acetaminophen standard solution with the HPSAM was performed by measuring the absorbance at 258 and 281 nm for each sample solution. These two compounds can be determined simultaneously in the concentration range 5.0-100 and 1.0-20 µg/ml, respectively. The procedure was repeated for some mixtures to show the applicability of the method.

RESULTS AND DISCUSSION

Preliminary Investigations
The absorption spectra of tramadol and acetaminophen are shown in Fig. 1. As can be seen, the spectra of the two compounds overlap, and each compound interferes in the spectrophotometric determination of the other. Therefore, the simultaneous determination of tramadol and acetaminophen is impossible by classical spectrophotometry. However, the system is suitable for simultaneous determination of tramadol and acetaminophen using the HPSAM.

Applying HPSAM
For the selection of the appropriate wavelengths for the application of the HPSAM, the following principles were followed. At two selected wavelengths, the analyte
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signals must be linear with the concentration; the interferent signals must remain equal, even if the analytical concentrations are changed. Also, the analytical signals of the mixture composed from the analyte and the interferent should be equal to the sum of the individual signals of the two compounds. In addition, the slope difference of the two straight lines obtained at $\lambda_1$ and $\lambda_2$ must be as large as possible in order to get good accuracy and sensitivity.

By considering Fig. 1, the absorbance corresponding to acetaminophen at two selected wavelengths $\lambda_1 = 258$ and $\lambda_2 = 281$ nm, are $b$ and $A_0$, respectively, while the absorbance corresponding to tramadol under the same conditions are $b'$ and $A'$ are equal. The following equations show the relation between them:

\[ A_{\lambda_1} = b_0 + b + M_{\lambda_1}C_{acetaminophen} \]  \( (1) \)

\[ A_{\lambda_2} = A_0 + A' + M_{\lambda_2}C_{acetaminophen} \]  \( (2) \)

where $A_{\lambda_1}$ and $A_{\lambda_2}$ are the analytical signals measured at 258 and 281 nm, respectively; $b_0$ and $A_0$ ($b_0 \neq A_0$) are the original analytical signals of acetaminophen $\lambda_1$ and $\lambda_2$, respectively; $b$ and $A'$ are the analytical signals of tramadol at $\lambda_1$ and $\lambda_2$, respectively; $M_{\lambda_1}$ and $M_{\lambda_2}$ are the slopes of the standard addition calibration lines at 258 and 281 nm, respectively. The two straight lines obtained intersect at the so-called H-point (-CH, AH) (Fig. 2).

At H-point, since $A_{\lambda_1} = A_{\lambda_2}$, $C_{acetaminophen} = -C_{H}$ from Eqs (1) and (2) it follows that:

\[ b_0 + b + M_{\lambda_1}(-C_{H}) = A_0 + A' + M_{\lambda_2}(-C_{H}) \]  \( (3) \)

\[ -C_{H} = \frac{(A_0-b_0) + (A'-b)}{(M_{\lambda_1} - M_{\lambda_2})} \]  \( (4) \)

$A'$ and $b$ are equal, hence

\[ C_{acetaminophen} = -C_{H} = \frac{(A_0-b_0)}{(M_{\lambda_1} - M_{\lambda_2})} \]  \( (5) \)

Substitution of $C_{acetaminophen}$ into Eqs 1 and 2 yields $A_{H}=b$ and the overall equation for the absorbance at H-point simplified to:

\[ A' = b = A_{tramadol} \]  \( (6) \)

The concentration of tramadol was calculated from the analytical signal from a calibration graph (Table 1).

**Accuracy and Precision of the Method**

Under the optimum conditions, simultaneous determination of different binary mixtures of tramadol and acetaminophen was made using the HPSAM. Regarding the tabulated results in Table 2, the accuracy of the results is satisfactory when the concentration of the tramadol and acetaminophen vary in the ranges of 5.0-100 and 1.0-20 µg/ml, respectively. To check the reproducibility of the method, five replicate experiments were performed and the relative standard deviations (RSD) were obtained for the mixtures. The results are given in Table 3. The precision of the results is satisfactory.

**Application**

In order to test the reliability of the proposed method, it was applied to the determination of tramadol and acetaminophen in pharmaceutical formulations. The results are presented in Table 4. The good agreement between these results and certificate values indicates the successful applicability of the proposed method for simultaneous determination of tramadol and acetaminophen in pharmaceutical formulations.

**CONCLUSION**

The HPSAM could be used for simultaneous spectrophotometric determination of tramadol and acetaminophen. The results of this study clearly show the potential and versatility of this method, which could be applied to evaluating tramadol and acetaminophen in pharmaceutical formulations. Finally, in comparison with chemometric methods, the H-point standard additions method (HPSAM) is very simple and mathematical software programs and additional information are not necessary.
Table 1. Characteristics of calibration graphs for the determination of tramadol and acetaminophen

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Wavelength (nm)</th>
<th>Slope (ml/µg)</th>
<th>Intercept</th>
<th>Correlation coefficient (n = 10)</th>
<th>Range (µg/ml)</th>
<th>Limit of detection* (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tramadol</td>
<td>258 or 281</td>
<td>0.00147</td>
<td>0.041</td>
<td>0.999</td>
<td>5.0-100</td>
<td>2.0</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>243</td>
<td>0.0754</td>
<td>0.0507</td>
<td>0.998</td>
<td>1.0-20</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Limit of detection was defined as \( C_L = 3S_b/m \) where \( C_L \), \( S_b \), and \( m \) are limit of detection, standard deviation of the blank signal and slope of the calibration graph, respectively.25

Table 2. Results obtained by the HPSAM for mixtures of tramadol and acetaminophen

<table>
<thead>
<tr>
<th>A-C equation</th>
<th>r (n=6)</th>
<th>Presented in sample (µg/ml)</th>
<th>Founda(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tramadol</td>
<td>Acetaminophen</td>
</tr>
<tr>
<td>( A_{258} = 0.1692 + 0.0454C_i )</td>
<td></td>
<td>0.9988</td>
<td>0.9991</td>
</tr>
<tr>
<td>( A_{281} = 0.1009 + 0.0127C_i )</td>
<td></td>
<td>0.9991</td>
<td>0.9984</td>
</tr>
<tr>
<td>( A_{258} = 0.3120 + 0.0449C_i )</td>
<td></td>
<td>0.9994</td>
<td>0.9993</td>
</tr>
<tr>
<td>( A_{281} = 0.1837 + 0.0136C_i )</td>
<td></td>
<td>0.9993</td>
<td>0.9993</td>
</tr>
<tr>
<td>( A_{258} = 0.4214 + 0.0440C_i )</td>
<td></td>
<td>0.9990</td>
<td>0.9990</td>
</tr>
<tr>
<td>( A_{281} = 0.2481 + 0.0108C_i )</td>
<td></td>
<td>0.9985</td>
<td>0.9983</td>
</tr>
<tr>
<td>( A_{258} = 0.5321 + 0.0502C_i )</td>
<td></td>
<td>0.9995</td>
<td>0.9993</td>
</tr>
<tr>
<td>( A_{281} = 0.3231 + 0.0148C_i )</td>
<td></td>
<td>0.9990</td>
<td>0.9990</td>
</tr>
</tbody>
</table>

Values of recovery are given in parentheses

Table 3. Results of five replicate experiments for the analysis of tramadol-acetaminophen mixtures.

<table>
<thead>
<tr>
<th>A-C equation</th>
<th>r(n=6)</th>
<th>Present in sample (µg/ml)</th>
<th>Founda (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tramadol</td>
<td>Acetaminophen</td>
</tr>
<tr>
<td>( A_{258} = 0.3414 + 0.0440C_i )</td>
<td>0.9988</td>
<td>0.9991</td>
<td>50</td>
</tr>
<tr>
<td>( A_{281} = 0.1721 + 0.0108C_i )</td>
<td>0.9991</td>
<td>0.9984</td>
<td>50</td>
</tr>
<tr>
<td>( A_{258} = 0.4079 + 0.0453C_i )</td>
<td>0.9991</td>
<td>0.9984</td>
<td>50</td>
</tr>
<tr>
<td>( A_{281} = 0.2452 + 0.0121C_i )</td>
<td>0.9984</td>
<td>0.9993</td>
<td>50</td>
</tr>
<tr>
<td>( A_{258} = 0.3459 + 0.0438C_i )</td>
<td>0.9994</td>
<td>0.9993</td>
<td>50</td>
</tr>
<tr>
<td>( A_{281} = 0.1733 + 0.0106C_i )</td>
<td>0.9993</td>
<td>0.9985</td>
<td>50</td>
</tr>
<tr>
<td>( A_{258} = 0.3519 + 0.0463C_i )</td>
<td>0.9999</td>
<td>0.9998</td>
<td>50</td>
</tr>
<tr>
<td>( A_{281} = 0.1826 + 0.0131C_i )</td>
<td>0.9998</td>
<td>0.9983</td>
<td></td>
</tr>
</tbody>
</table>

Mean

Standard deviation

RSD(%)
Table 4. Determination of tramadol and acetaminophen in pharmaceutical formulations by proposed method.

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Certified value (mg)</th>
<th>Found (µg/g)</th>
<th>Error(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tramadol</td>
<td>Acetaminophen</td>
<td>Tramadol</td>
</tr>
<tr>
<td>Tramadol</td>
<td>100</td>
<td>-</td>
<td>97.5±1.76</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>-</td>
<td>325</td>
<td>-</td>
</tr>
<tr>
<td>*Combined dosage form</td>
<td>37.5</td>
<td>325</td>
<td>36.1±1.12</td>
</tr>
<tr>
<td>**Combined dosage form</td>
<td>50</td>
<td>500</td>
<td>38.6±1.3</td>
</tr>
</tbody>
</table>

*This combined formulation contain: Acetaminophen, 325 mg and tramadol, 37.5 mg (corresponding to Tramacet and Ultracet tablets).

**This combined formulation contain: Acetaminophen, 500 mg and tramadol, 50 mg (corresponding to Instrel.tablet).

Figure 1. Absorption spectra of 5.0 µg/ml tramadol and 50 µg/ml acetaminophen.
Figure 2. Plot of HPSAM for simultaneous determination of the mixture of tramadol-acetaminophen, Conditions: tramadol, 5.0 µg/ml; acetaminophen, 50 µg/ml.

REFERENCES

Simultaneous Spectrophotometric...  Ali Reza Zarei*, Kobra Mardi, Soheila Chalavi
Acetaminophen and Tramadol. Method of adding.

H DATABASE LINK

Title


Abstract

The objective of this study was to develop a simple, precise and accurate method for the simultaneous determination of Acetaminophen and Tramadol in pharmaceutical preparations. HPLC method was used to this purpose. The procedure was validated using the ICH guidelines. The results showed that the method was suitable for the determination of the two compounds. The results were compared with the results of the literature.

Keywords: Determination of the compounds, HPLC method.