Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND TAPENTADOL HYDROCHLORIDE IN BULK DRUG AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Paracetamol and Tapentadol hydrochloride in bulk and in pharmaceutical dosage form. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Paracetamol and Tapentadol hydrochloride show an isoabsorptive point at 225 nm in methanol. The second wavelength used is 274 nm, which is the λ-max of Tapentadol hydrochloride in methanol. The linearity was obtained in the concentration range of 4-12 µg/ml for Paracetamol and 30-70 µg/ml Tapentadol hydrochloride. The concentrations of the drugs were determined by using ratio of absorbance at isoabsorptive point and at the λ-max of Tapentadol hydrochloride. The method was successfully applied to pharmaceutical dosage form because of no interference. The results of analysis have been validated by recovery studies.

KEY WORDS

Paracetamol, Tapentadol hydrochloride, Methanol, Q-Absorbance Ratio method, Isoabsorptive point.

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INTRODUCTION

Paracetamol (PCM) is chemically 4-hydroxyacetanilide (Figure 1-A) used as analgesic and antipyretic.\textsuperscript{[1]} Paracetamol acts primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2 and COX-3 enzymes involved in prostaglandin (PG) synthesis.\textsuperscript{[2]}

Tapentadol \textsuperscript{[4],[ 7-9]} is a novel centrally acting analgesic. It has structural similarities to tramadol. The drug has a unique mode of action in that it functions as an agonist at the $\mu$-opioid receptor, and as a norepinephrine reuptake inhibitor. This dual mode of action provides analgesia at similar levels of more potent narcotic analgesics such as hydrocodone, oxycodone and morphine, but with a more tolerable side effect profile. The chemical name is 3 - [(1\textit{R},2\textit{R}) -3 - (dimethylamino) –1 - ethyl-2 -methylpropyl] phenol monohydrochloride.

The therapeutic importance of these two compounds justifies establishing analytical methods for its determination in bulk and pharmaceutical dosage form.

The chemical structures of Paracetamol and Tapentadol are shown in Figure 1 (A), (B). \textsuperscript{[1, 4]}

\textbf{Fig.1: Chemical structure of (A) Paracetamol and (B) Tapentadol hydrochloride.}

Paracetamol is official in IP, BP and USP and is estimated by UV-Visible Spectrophotometric method as per IP, USP and BP. \textsuperscript{[3, 5, 6]} In BP a redox titration for PCM is given for drug substance.\textsuperscript{[6]} Literature review also reveals HPLC, UV spectrophotometric and HPTLC method for the estimation of PCM with other drugs. \textsuperscript{[7, 8, 9]} Literature survey does not reveal any simple spectrophotometric method of Paracetamol and Tapentadol hydrochloride in bulk or Pharmaceutical dosage form. So the objective of this work was to develop simple, precise and rapid spectrophotometric methods for combined dosage form containing Paracetamol, and tapentadol.

MATERIALS AND METHODS

Instrumentation

Double beam UV-visible spectrophotometer (heλios Alpha, Model - UV A 1002E) having two matched quartz cells with 1 cm light path. An Electronic analytical balance (Contech, CA34 Model) was used in the study.
Material and reagent

Paracetamol (PCM) was obtained from Smt. R. D. Gardi B. Pharmacy College, Rajkot and Tapentadol hydrochloride (TP) bulk powder was kindly gifted by Ami life science, Vadodara, India.

Preparation of Standard Stock solution of PCM and TP:

Accurately weighed quantity 100 mg of PCM and TP were transferred into separate 100 ml volumetric flask, dissolved and diluted up to mark with Methanol (100 ml). This will give a stock solution having strength of 1000 μg/ml of each.

Preparation of Working Standard Solution of PCM and TP:

100 μg/ml of PCM and TP solution were prepared by diluting 10 ml of stock solution to 100 ml with methanol in separate 100 ml volumetric flask.

Suitable aliquots of this solution were diluted up to the mark with methanol to get the concentration range of 4, 6, 8, 10 and 12 μg/ml for PCM and 30, 40, 50, 60 and 70 μg/ml for TP.

Selection of analytical wavelength:

From working standard solution of PCM (100 μg/ml) and PAM (100 μg/ml), prepare 15 μg/ml for PCM and TP both. The scanning of solution of PCM and TP were carried out in the range of 200–400 nm against using methanol as a blank. The maximum absorption (λmax) of TP was found at 274 nm and iso-absorptive point at 225 nm. Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths.

Preparation of calibration curve:

Standard solutions of PCM in the concentration range of 4 to 12 μg/ml obtained by transferring (0.4, 0.6, 0.8, 1.0 and 1.2 ml) of PCM stock solution (100 μg/ml) to the series of 10 ml volumetric flasks and standard solutions of TP in the concentration range of 30 to 70 μg/ml were obtained by transferring (3.0, 4.0, 5.0, 6.0 and 7.0 ml) of TP stock solution (100 μg/ml) to the series of 10 ml volumetric flasks. Then volume was adjusted up-to mark with methanol. All dilutions were scanned in wavelength range of 200 nm to 400 nm. The absorbance was plotted against the respective concentrations to obtain the calibration curves.

METHODOLOGY

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the λmax of one of the two components. From the overlain spectra of two drugs (as shown in figure 2), it shows that PCM and TP having iso-absorptive point at 225 nm. The second wavelength used is 274 nm, which is the λmax of TP.
Figure-2: Overlain Spectrum of Paracetamol and Tapentadol hydrochloride in methanol.

Working standard solutions having concentration 4, 6, 8, 10 and 12 μg/ml for TP and 30, 40, 50, 60 and 70 μg/ml for TP were prepared and the absorbance at 225 nm (iso-absorptive point) and 274 nm (λmax of TP) were measured and absorptivity coefficient were calculated using calibrations curve.

A set of two equations were framed using the mean absorptivity.

\[
C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \cdot \frac{A_1}{a_x_1}
\]

\[
C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \cdot \frac{A_1}{a_y_1}
\]

\[
Q_m = \frac{\text{Absorbance of sample solution at 257nm (A2)}}{\text{Absorbance of sample solution at 291 nm (A1)}}
\]

\[
Q_x = \frac{\text{Absorptivity of PCM at 257 nm}}{\text{Absorptivity of PCM at 291 nm}}
\]

\[
Q_y = \frac{\text{Absorptivity of PAM at 257 nm}}{\text{Absorptivity of PAM at 291 nm}}
\]

Where, Qx and Qy are value of PCM and TP respectively, ax₁ and ay₁ are absorptivity value at iso-absorptive point for PCM and TP.
VALIDATION PARAMETERS

Validation of developed method was carried out as per ICH guideline. Parameters such as Linearity and range, Accuracy, Precision, LOD and LOQ were taken up as tests for analytical method validation.

**Linearity and Range:**

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity responses was determined in the range of 4-12 μg/ml for PCM and 30-70 μg/ml for TP. Plot the calibration curve of absorbance verses concentration at specified wavelength and determine correlation coefficient and regression equations for PCM and TP.

**Precision**

Precision of the method was determined in terms of Repeatability, Intraday and Interday precision. Repeatability (% RSD) was assessed by analyzing test drug solution within the calibration range, six times on the same day. Intraday variation (% RSD) was determined by analysis of this solution three times on the same day. Interday precision (% RSD) was determined by analysis of this solution on three different days.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y-intercept) and S, is the mean of the slope of calibration plot.

**Recovery Studies:**

Recovery studies were done so as to check the accuracy of the method. Known amounts of standard solutions of PCM and TP were added to pre-quantified sample solutions of PCM and TP and absorbance were determined at 225 nm and 274 nm. Concentration of the drug in the mixture was calculated using the equations. The analysis was done in a set of 3 replicates.

**Table-1 Result of Recovery Studies for PCM in pharmaceutical dosage form:**

<table>
<thead>
<tr>
<th>Amount of PCM in tablet (μg/ml)</th>
<th>Amount of Std PCM added (μg/ml)</th>
<th>Total amount of PCM (μg/ml)</th>
<th>Total amount of PCM found (μg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>6.4</td>
<td>14.4</td>
<td>14.478±0.1543</td>
<td>99.16</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>16</td>
<td>15.92±0.1590</td>
<td>99.50</td>
</tr>
<tr>
<td>8</td>
<td>9.6</td>
<td>17.6</td>
<td>17.51±0.1184</td>
<td>99.47</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]
Table 2: Result of Recovery Studies for TP in pharmaceutical dosage form.

<table>
<thead>
<tr>
<th>Amount of TP in Tablet (μg/ml)</th>
<th>Amount of Std TP added (μg/ml)</th>
<th>Total amount of TP (μg/ml)</th>
<th>Total amount of TP found (μg/ml) Mean* ± SD</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>18</td>
<td>17.82±0.1401</td>
<td>99.02</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>20</td>
<td>19.83±0.1408</td>
<td>99.17</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>22</td>
<td>21.76±0.1889</td>
<td>98.89</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]

Application of Proposed Method to Pharmaceutical dosage form:

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 325mg of Paracetamol and 50mg of Tapentadol into a 100 ml volumetric flask, added 50 ml of methanol and mechanically stirred for 20 minutes. This solution was filtered through the Whatmann filter paper No. 41 and residues were washed with methanol. The filtrate and washings were combined and volume was made-up to 100 ml with methanol. 0.2 ml from above stock solution is transferred to 100 ml volumetric flask and dilute to 100 ml with methanol to get final concentration as 6.5 ppm of Paracetamol and 1 ppm of Tapetadol.

Absorbance of the resulting solution was measured at 225 nm and 274 nm against methanol.

Table 3: Analysis of PCM and TP in Pharmaceutical dosage form:

<table>
<thead>
<tr>
<th>Synthetic mixture</th>
<th>Label claim(mg)</th>
<th>PCM %Recovery ± SD (% of label claim*)</th>
<th>PAM</th>
<th>PCM</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>99.28±0.0450</td>
<td>50</td>
<td>99.33±0.0125</td>
<td></td>
</tr>
</tbody>
</table>

[*=mean value of 5 determination]

RESULTS AND DISCUSSION

Absorption Maxima:

Iso-absorptive wavelength of PCM (15 ppm) and TP (15 ppm) were recorded as 225nm (λ₁) and overlain spectra were recorded in Fig: 2. 274 nm wavelength was used as λ₂. Regression characteristics for PCM and TP are shown in Table 4.
Table 4: Regression Characteristics of Paracetamol and Tapentadol:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PCM</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>225(λ₁)</td>
<td>274(λ₂)</td>
</tr>
<tr>
<td></td>
<td>225(λ₁)</td>
<td>274 (λ₂)</td>
</tr>
<tr>
<td>Linearity (μg/ml)</td>
<td>4-12</td>
<td>4-12</td>
</tr>
<tr>
<td></td>
<td>30-70</td>
<td>3-700</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>y = 0.0223x + 0.0034</td>
<td>y = 0.0155x - 0.0274</td>
</tr>
<tr>
<td></td>
<td>y = 0.0184x + 0.0601</td>
<td>y = 0.0088x - 0.0109</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0223</td>
<td>0.0155</td>
</tr>
<tr>
<td></td>
<td>0.0184</td>
<td>0.0088</td>
</tr>
<tr>
<td>r²</td>
<td>0.9944</td>
<td>0.9985</td>
</tr>
<tr>
<td></td>
<td>0.9965</td>
<td>0.9995</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0034</td>
<td>0.0274</td>
</tr>
<tr>
<td></td>
<td>0.0601</td>
<td>0.0109</td>
</tr>
<tr>
<td>S.D. of Intercept</td>
<td>0.0006</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>0.0144</td>
<td>0.0061</td>
</tr>
</tbody>
</table>

Method Validation:

The linearity range for PCM and TP were 4-12 μg/mL and 3-700 μg/mL respectively. Recovery studies was carried out by addition of standard drug solution to pre-analyzed pharmaceutical dosage form solution at three different concentration levels taking into consideration percentage purity of added bulk drug sample. The results of the recovery studies are found to be satisfactory for PCM and TP and shown in Table 1 and 2 respectively. The result of assay procedure obtained was showed in Table 3. Summary of Other validation parameters including Repeatability, Intraday, Interday, LOD and LOQ were found to be satisfactory and are shown in Table 5.

Table 5: Validation results of Paracetamol and Tapentadol:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCM</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength(nm)</td>
<td>225(λ₁)</td>
<td>274(λ₂)</td>
</tr>
<tr>
<td></td>
<td>225(λ₁)</td>
<td>274 (λ₂)</td>
</tr>
<tr>
<td>Repeatability(%RSD)</td>
<td>0.6747</td>
<td>1.8423</td>
</tr>
<tr>
<td>(n=6)</td>
<td>1.4045</td>
<td>2.0236</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td>0.6143-1.3364</td>
<td>1.7462-1.9915</td>
</tr>
<tr>
<td></td>
<td>0.1352-0.8005</td>
<td>0.4734-1.0365</td>
</tr>
</tbody>
</table>
**CONCLUSION**

The results obtained by applying the suggested procedures, it is proved that the proposed method is accurate, precise, simple, sensitive, selective and rapid and can be applied successfully in routine analysis for the estimation of PCM and TP in bulk and in pharmaceutical dosage form. The developed method was validated as par ICH guidelines.

**ACKNOWLEDGEMENT**

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**REFERENCES**


