Development and validation of UV spectrophotometric methods for simultaneous estimation of Paracetamol and Ibuprofen in pure and tablet dosage form

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ABSTRACT

Two simple, rapid, accurate, precise, and economic spectrophotometric methods for simultaneous estimation of paracetamol and ibuprofen in pure and tablet dosage form have been developed. Method I is based on solving simultaneous equation. Paracetamol and ibuprofen show absorbance maximums at 256 and 222.4 nm respectively, so absorbance was measured at the same wavelengths for the estimation of paracetamol and ibuprofen. Method II is based on determination of Q-value. Absorbance is measured at 226.4 nm (Isoabsorptive point) and 222.4nm ($\lambda_{max}$ of ibuprofen). Both drugs obey the Beer Lambert’s law in the concentration range of 5-30 µg/mL. Methods are validated according to ICH guidelines and can be adopted for the routine analysis of paracetamol and ibuprofen in pure and tablet dosage form.

Key words: Paracetamol, Ibuprofen, Simultaneous equation, Absorbance ratio, Validation.

INTRODUCTION

Chemically, paracetamol is [N-(4-hydroxyphenyl)acetamide]. It is an analgesic antipyretic agent. It is effective in treating mild to moderate pain such as headache, neuralgia and pain of musculo-skeletal origin [1, 2]. The most recent methods for determination of paracetamol included chromatographic [3-5], electrochemical [6-8] and spectrophotometric [9-11] techniques.

The 2-arylpropionic acid derivative, Ibuprofen [RS-2-(4-isobutyl-phenyl)propionic acid], is one of the most potent orally active antipyretic, analgesic and nonsteroidal anti-inflammatory drug (NSAID) used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions. Ibuprofen is characterized by a better tolerability compared with
other NSAIDs. The techniques most recent used for determination of ibuprofen included chromatographic [12-15], electrochemical [16,17] and spectrophotometric [18-23] methods.

Method validation is an important issue in pharmaceutical analysis. It confirms that the analytical procedure employed for the analysis is suitable and reliable for its intended use. In present study, all validation parameters for quantitative analysis of paracetamol and ibuprofen in tablets were tested and data were evaluated according to their acceptance criteria.

The review of literature revealed that the combined dosage form has been estimated by spectrophotometric method by using methanol as solvent in soft gelatin capsules [24] but no method is yet reported in solvent 0.1N NaOH. Furthermore methanol being volatile in nature creates problem in accuracy while 0.1 N NaOH being aqueous in nature serves for the accuracy. Also methanol is more expensive than NaOH. Thus, this method is more accurate and cost effective. This paper describes two simple, rapid, accurate, precise and economical methods for simultaneous determination of paracetamol and ibuprofen in tablet dosage form.

**MATERIALS AND METHODS**

**Instruments**

UV-visible double beam spectrophotometer, Systronic model 2201 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used and Shimadzu AY220 balance was used for weighing.

**Materials**

Pure paracetamol was kindly gifted from Zest pharma, Indore and ibuprofen from Wanbury ltd. Navi Mumbai. The commercially available tablets, Combiflam (Label claim: paracetamol-325 mg, ibuprofen-400 mg) was procured from local market. All the chemicals and reagents were of analytical grade.

**Selection of common solvent**

After assessing the solubility of drugs in different solvents 0.1N NaOH has been selected as common solvent for developing spectral characteristics.

**Selection of wavelength**

A representative overlain spectrum of Ibuprofen and Paracetamol in 0.1N NaOH is shown in Fig 1. The dilution was obtained to the concentration of 10 µg/ml for both paracetamol and ibuprofen solution. Both the solutions were scanned in UV range (200-400nm) in 10 mm cell against solvent blank. The study of spectrum revealed that paracetamol show a well defined $\lambda_{max}$ at 256 nm whereas ibuprofen shows at 222.4 nm. These two wavelengths were selected for development of simultaneous equation. From the overlain spectrum, it is evident that isoabsorptive point is at 226.4 nm for absorbance ratio method.

**Preparation of standard stock solution and Study of Beer-Lambert’s Law**

The standard stock solutions of paracetamol and ibuprofen were prepared by dissolving 0.025 gm of each drug in 0.1N NaOH and final volume was adjusted with same solvent in 100 mL of
volumetric flask to get a solution containing 250 µg/mL of each drug. Aliquots of working stock solutions of paracetamol and ibuprofen were diluted with 0.1N NaOH solution to get concentration in range of 5-30 µg/mL for both the individual drug. The absorbances of resulting solutions were measured at their respective max and isobestic point. A calibration curve as concentration vs. absorptivity (Fig-3) to study the Beer-Lambert’s Law and regression equation. concentration vs. absorbance (Fig-2) was constructed and by calculating absorptivity of both solutions were measured at their respective max and isobestic point. A calibration curve as concentration in range of 5-30 µg/mL for both the individual drug. The absorbances of resulting solutions of paracetamol and ibuprofen were diluted with 0.1N NaOH solution to get volumetric flask to get a solution containing 250 µg/mL of each drug. Aliquots of working stock solutions of paracetamol and ibuprofen were diluted with 0.1N NaOH solution to get concentration in range of 5-30 µg/mL for both the individual drug. The absorbances of resulting solutions were measured at their respective max and isobestic point. A calibration curve as concentration vs. absorptivity (Fig-3) to study the Beer-Lambert’s Law and regression equation. concentration vs. absorbance (Fig-2) was constructed and by calculating absorptivity of both solutions were measured at their respective max and isobestic point. A calibration curve as concentration in range of 5-30 µg/mL for both the individual drug. The absorbances of resulting solutions of paracetamol and ibuprofen were diluted with 0.1N NaOH solution to get concentration in range of 5-30 µg/mL for both the individual drug. The absorbances of resulting solutions were measured at their respective max and isobestic point. A calibration curve as concentration vs. absorptivity (Fig-3) to study the Beer-Lambert’s Law and regression equation.

Method I (Simultaneous equation method)
If a sample contain two absorbing drug each of which absorbs at the \( \lambda_{\text{max}} \) of the other, it may be possible to determine both drugs by the technique of simultaneous equation.

Two wavelengths selected for the development of the simultaneous equations are 256 nm and 222.4 nm. The absorptivity values determined for paracetamol are 0.0643 (ax1), 0.0242 (ax2) and for ibuprofen are 0.0055 (ay1), 0.0351 (ay2) at 256 nm and 222.4 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs.

\[
\begin{align*}
C_x &= \frac{A_2 \times 0.0643 - A_1 \times 0.0242}{-0.0242} & \text{Eqn.1} \\
C_y &= \frac{A_1 \times 0.0242 - A_2 \times 0.0643}{-0.0242} & \text{Eqn.2}
\end{align*}
\]

Where \( C_{\text{paracetamol}} \) and \( C_{\text{ibuprofen}} \) are concentration of paracetamol and ibuprofen respectively in µg/mL. \( A_1 \) and \( A_2 \) are the absorbance of the mixture at 256 nm and 222.4 nm respectively.

Method II (Absorbance ratio method)
Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isoabsorptive point and the other being the wavelength of maximum absorption of one drug. From overlain spectra (Fig. 1), 222.4 nm (\( \lambda_{\text{max}} \) of ibuprofen) and 226.4 nm (isoabsorptive point) are selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorptivity values determined for paracetamol are 0.0242 (ax1), 0.0294 (ax2) and for ibuprofen are 0.0351 (ay1), 0.0253 (ay2) at 222.4 nm and 226.4 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of drugs.

\[
\begin{align*}
C_x &= \frac{A_{11} - 0.7207}{0.4980} \times \frac{\Delta l}{0.0294} & \text{Eqn.3} \\
C_y &= \frac{A_{12} - 1.3137}{-0.4290} \times \frac{\Delta l}{0.0253} & \text{Eqn.4}
\end{align*}
\]

Where \( C_{\text{paracetamol}} \) and \( C_{\text{ibuprofen}} \) are concentration of paracetamol and ibuprofen respectively in µg/mL. \( A_1 \) and \( A_2 \) were the absorbance of the sample at 222.4 nm and 226.4 nm respectively.
**Analysis of the tablet formulations**

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of paracetamol was transferred to 100 mL volumetric flask and dissolved in 0.1N NaOH and final volume was made up with 0.1N NaOH. The sample solution was then filtered through Whatman filter paper No.41. From the above solution 10 mL of solution was taken and diluted to 50 mL with 0.1N NaOH to get a solution containing 100 µg/mL of paracetamol and corresponding concentration of ibuprofen. From above, 0.65 mL of solution was diluted with same solvent in 10 mL volumetric flask to get final concentration of paracetamol 6.5µg/mL and ibuprofen 8µg/mL. Analysis procedure was repeated six times with tablet formulation. The results of tablet analysis are reported in Table 2.

**Validation of the developed methods**

**Linearity**

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method I and II, the Beer-Lambert’s concentration range was found to be 5-30 µg/mL for both drugs. The linearity data for both methods are presented in Table 1.

**Accuracy**

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The result of the recovery studies are reported in table 3.

**Precision:**

**Repeatability**

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The results of statistical evaluation are given in Table 2.

**Intermediate Precision (Interday and Intraday precision)**

The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table 4.

**RESULT AND DISCUSSION**

Linearity range for paracetamol and ibuprofen are 5-30 µg/mL at respective selected wavelengths. The coefficient of correlation for paracetamol at 256 nm and for ibuprofen at 222.4 nm is 1 and 0.999 respectively. Both drugs shows good regression values at their respective wavelengths and the results of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

Percentage estimation of paracetamol and ibuprofen from tablet dosage form by method I is 98.98 and 99.50 and by method II is 98.69 and 99.68 respectively with standard deviation <2 (Table 2).
Table No. 1: Optical parameters and regression characteristic

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Paracetamol at ( \lambda_{\text{max}} )</th>
<th>Ibuprofen at ( \lambda_{\text{max}} )</th>
<th>Paracetamol at iso-absorptive point</th>
<th>Ibuprofen at iso-absorptive point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s Law range</td>
<td>5-30 ( \mu g/mL )</td>
<td>5-30 ( \mu g/mL )</td>
<td>5-30 ( \mu g/mL )</td>
<td>5-30 ( \mu g/mL )</td>
</tr>
<tr>
<td>Regression Equation (Y)</td>
<td>( Y = 0.064x )</td>
<td>( Y = 0.034x )</td>
<td>( Y = 0.029x )</td>
<td>( Y = 0.0254x )</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.064</td>
<td>0.034</td>
<td>0.029</td>
<td>0.0254</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.001</td>
<td>0.003</td>
<td>0.004</td>
<td>0.0002</td>
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<tr>
<td>Correlation coefficient</td>
<td>1</td>
<td>0.999</td>
<td>0.999</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

Table No. 2: Analysis of Tablet Formulation

<table>
<thead>
<tr>
<th>Absorbance Reading at selected wavelength</th>
<th>Conc. Acquired</th>
<th>% Found (( \mu g/ml ))</th>
<th>Method I and II</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td>256nm</td>
<td>Par 6.5</td>
<td>98.54</td>
<td>Par 98.31</td>
<td>98.50</td>
<td>98.30</td>
</tr>
<tr>
<td></td>
<td>IBU 8.5</td>
<td>98.21</td>
<td>IBU 98.86</td>
<td>98.81</td>
<td>98.49</td>
</tr>
<tr>
<td></td>
<td>Par 6.5</td>
<td>98.53</td>
<td>Par 100.24</td>
<td>98.05</td>
<td>100.69</td>
</tr>
<tr>
<td></td>
<td>IBU 8.5</td>
<td>100.18</td>
<td>IBU 100.56</td>
<td>99.12</td>
<td>101.16</td>
</tr>
<tr>
<td></td>
<td>Par 6.5</td>
<td>99.95</td>
<td>Par 100.81</td>
<td>99.44</td>
<td>100.83</td>
</tr>
<tr>
<td></td>
<td>IBU 8.5</td>
<td>99.22</td>
<td>IBU 98.23</td>
<td>99.22</td>
<td>98.62</td>
</tr>
</tbody>
</table>

| Average | Par 98.98 | 99.50 | 98.69 | 99.68 |
| S.D.    | 0.8496   | 1.1684 | 0.4446 | 1.3399 |
| COV     | 0.8584   | 1.1742 | 0.4505 | 1.3442 |
| SE      | 0.3469   | 0.4770 | 0.1815 | 0.5470 |

Table No. 3: Result of recovery study

<table>
<thead>
<tr>
<th>Amount Taken (( \mu g/ml ))</th>
<th>PAR</th>
<th>IBU</th>
<th>PAR</th>
<th>IBU</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>8.0</td>
<td>6.5</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
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<tr>
<td>12.0</td>
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<td>12.0</td>
<td>12.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount Added (( \mu g/ml ))</th>
<th>%</th>
<th>80</th>
<th>100</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>6.5</td>
<td>6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Recovery*</th>
<th>99.89 ±0.82</th>
<th>99.00 ±1.14</th>
<th>100.02 ±0.78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Recovery ±S.D.</td>
<td>99.63±0.91</td>
<td>98.99±0.94</td>
<td>99.5±1.29</td>
</tr>
</tbody>
</table>

Table No. 4: Validation parameters

<table>
<thead>
<tr>
<th>Method</th>
<th>Drug</th>
<th>Precision (% COV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intraday n=3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>First day</td>
</tr>
<tr>
<td>I</td>
<td>PAR</td>
<td>0.5621</td>
</tr>
<tr>
<td></td>
<td>IBU</td>
<td>0.9143</td>
</tr>
<tr>
<td>II</td>
<td>PAR</td>
<td>0.7025</td>
</tr>
<tr>
<td></td>
<td>IBU</td>
<td>0.6924</td>
</tr>
</tbody>
</table>

The validity and reliability of proposed methods are assessed by recovery studies. Sample recovery for both the methods is in good agreement with their respective label claims, which suggest non interference of formulation additives in estimation (Table 3).

Precision is determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval of time and interassay precision. The standard deviation, coefficient of variance and standard error are calculated for paracetamol and ibuprofen. The results are mentioned in Table 2. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter day precision study for both the methods % COV are not more than 2.0% indicates good repeatability and intermediate precision (Table 4).
Fig.3 Response ratio curve A-Paracetamol at 256nm, B-Ibuprofen at 222.4nm, C-Paracetamol-226.4nm and D-Ibuprofen at 226.4nm

CONCLUSION

The proposed spectrophotometric methods are simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. These two methods can be successfully used for simultaneous estimation of paracetamol and ibuprofen in pure and tablet dosage form.

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The authors are greatly thankful to P.D.V.V.P.F’s College of Pharmacy, Ahmednagar, India for providing access to facilities and necessary infrastructure to carry out research work. We are also thankful to for providing us the free gift sample of Ibuprofen by Wanbury ltd. Navi Mumbai and Paracetamol by Zest pharma, Indore which were required for our research work.

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