DEVELOPMENT OF VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF IBUPROFEN, PARACETAMOL AND CAFFEINE IN THE BULK DRUG AND MARKETED FORMULATION

Minal Harde1,3*, Sagar Wankhede2, Pravin Chaudhari3

1Department of Pharmaceutical Sciences, JNTU, Hyderabad-500085.
2Pad. Dr. D.Y. Patil Institute of Pharmaceutical Science and Research, Sant Tukaram Nagar, Pimpri, Pune - 411018, Maharashtra, India.
3P. E Society’s Modern College of Pharmacy, Sector No. 21, Yamunanagar, Nigdi, Pune - 411044, Maharashtra, India.

ABSTRACT

A simple, sensitive, accurate, precise, and economic UV spectrophotometric method has been developed and validated for the simultaneous estimation of Ibuprofen, Paracetamol and Caffeine in tablet dosage form. Ibuprofen, Paracetamol and Caffeine showed maximum absorption at 221, 257 and 273 nm respectively using a UV–Visible spectrophotometer (Jasco, model V-630). Beer’s law was obeyed in the concentration range of 5-30 μg ml-1, 4-24 μg ml-1 and 1-20 μg/ ml for Ibuprofen, Paracetamol and Caffeine respectively with a correlation coefficient was found to be 0.999 for all drugs. The method was validated for various parameters according to ICH guidelines. The low relative standard deviation values indicate good precision and high recovery values indicate accuracy of the proposed method. Assay results were in good agreement with label claim.

KEYWORDS: Ibuprofen, Paracetamol, Caffeine, simultaneous equation method, UV spectrophotometric method.
INTRODUCTION

Ibuprofen (IBF) is chemically, RS-2-(4-isobutyl-phenyl)propionic acid, is one of the most potent orally active antipyretic, analgesic and non-steroidal anti-inflammatory drug (NSAID) used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions such as dysmenorrhea, headache including migraine, postoperative pain and dental pain. Its molecular formula is, C13H18O2 with a molecular mass of 206.29 g/mol.\(^1\)-\(^2\) Paracetamol (PCM) chemically is N-(4-hydroxyphenyl)acetamide (Figure 2) with molecular formula C8H9NO2 and has a molecular mass of 151.17 g/mol. It has analgesic and antipyretic properties and anti-inflammatory activity. Caffeine (CFN) chemically is 1, 3, 7-trimethyl-2, 3, 6, 7-tetrahydro-1H-purine-2,6-dione (Figure 3). It acts by inhibition of cyclic nucleotide phosphodiesterases. Caffeine in combination with IBF and PCM, causes reduction in the amount of prostaglandin whereas CFN is also known to increase the analgesic effect of PCM and IBU, synergistically, providing relief from symptoms like headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, migraine, general pain, toothache and menstrual pain. The combination is also found to be effective in controlling fever originating from bacterial or viral infection.\(^3\)-\(^9\)

![Figure 1: Ibuprofen](image1)

![Figure 2: Paracetamol](image2)

![Figure 3: Caffeine](image3)

Literature survey reveals several methods that have been used for the quantitative determination of these three drugs individually and in combination with other drugs\(^4\)-\(^9\). The
objective of present work is to develop a new UV spectrophotometric method for the simultaneous estimation of Ibuprofen, Paracetamol and Caffeine in pharmaceutical dosage form.

MATERIALS AND METHOD

Chemicals and reagents

Ibuprofen, Paracetamol and Caffeine pure powder were obtained as a gift samples from Emcure Pharmaceutical Limited, India. Marketed tablets were purchased from local pharmacy shop. Analytical grade Methanol was purchased from Merck (Darmstadt, Germany).

Instrument: Shimadzu UV–Visible spectrophotometer (Jasco, UV-630) was employed with a spectral band width of 1 nm and a wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells).

Selection of solvent and wavelength

Solubility of IBF, PCM and CFN were checked in suitable solvents like water and methanol. The UV spectrum was recorded after suitable dilution of drugs in water where methanol was used as a co-solvent. The three wavelengths 221, 257 and 273 nm (Figure 4) were obtained are the λmax of IBF, PCM and CFN respectively.

Figure 4. Overlain spectra of Ibuprofen, Paracetamol and Caffeine

Preparation Standard Stock Solutions

IBF, PCM and CFN (10 mg each) were weighed separately and transferred to 100 ml volumetric flask. 30 ml methanol was added and allowed for ultrasonication for 20 min. Then
volume was made upto the mark using distilled water as a solvent ethanol to get the final concentration of 100 μg/ ml.

**Application of Simultaneous equation method:**
Quantitative estimation of three components by Simultaneous equation method were employed in present method. The three selected wavelengths were 221 nm of IBF, 257 nm of PCM and 273 nm of CFN were used for estimation, at which three drugs showed maximum absorbance respectively. The concentrations of three drugs in the mixture were calculated using the following equations.

\[
C_{IBF} = \frac{(A1 (ay2az3-az2ay3) - ay1 (A2az3-az2A3) + az1 (A2ay3-ay2A3))}{ax1(ay2az3-az2ay3) - ay1(ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}
\]

\[
C_{PCM} = \frac{(ax1(A2az3-az2A3) - A1(ax2az3-az2ax3) + az1(ax2A3-A2ax3))}{ax1(ay2az3-az2ay3) - ay1(ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}
\]

\[
C_{CFN} = \frac{(ax1(ay2A3-A2ay3) - ay1(ax2A3-A2ax3) + A1(ax2ay3-ay2ax3))}{ax1(ay2az3-az2ay3) - ay1(ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}
\]

Where, \(C_{IBF}\), \(C_{PCM}\) and \(C_{CFN}\) term indicates the concentrations of IBF, PCM and CFN respectively. \(A_1\), \(A_2\) and \(A_3\) denotes the absorbances of sample at 221, 257 and 273 nm, respectively, \(ax_1\), \(ax_2\) and \(ax_3\) are the absorptivity of IBF at 221, 257 and 273 nm respectively, \(ay_1\), \(ay_2\) and \(ay_3\) are the absorptivity of PCM at 221, 257 and 273 nm respectively, \(az_1\), \(az_2\) and \(az_3\) are the absorptivity of CFN at 221, 257 and 273 nm, respectively.

**Analysis of marketed formulation**
For the analysis, 20 tablets were weighed and their average weight was determined. The tablets were then crushed to obtain fine powder and equivalent weight of tablet powder was transferred to 100 ml volumetric flask, dissolved in 30 ml of methanol and allowed to ultrasonicate for 20 min. Finally, the volume was made up to the mark with distilled water. The solution was then filtered through whatmann filter paper. From this solution, 1 ml was pipette out into a 10 ml volumetric flask and diluted with distilled water up to the mark. The absorbance of the above solution was measured at 221, 257 and 273 nm. The concentration of each analyte was estimated using the simultaneous equation.
RESULTS AND DISCUSSION
The analytical method was validated with respect to parameters such as linearity, precision, limit of detection (LOD), limit of quantitation (LOQ) and accuracy.

Linearity
Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 5-30 μg/ml for IBF, 4-24 μg/ml for PCM and 1-20 μg/ml for CFN (Figure 5-7). Absorbance were plotted versus their respective concentrations and linear regression analysis performed. Correlation coefficients (n=3) were found to be more than 0.999 for IBF, PCM and CFN. The regression data as given in Table 1, showed a good linear relationship on the resultant curves.

Table 1: Results of Linearity Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IBF</th>
<th>PCM</th>
<th>CFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>5-30 μg ml⁻¹</td>
<td>4-24 μg ml⁻¹</td>
<td>1-20 μg ml⁻¹</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope</td>
<td>0.035</td>
<td>0.056</td>
<td>0.049</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.027</td>
<td>0.027</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 5. Overlay spectra of Standard Ibuprofen (5-30 μg ml⁻¹)
Precision

To check the degree of repeatability of the method, suitable statistical evaluation was carried out. The concentrations of three drugs were measured six times on the same day at intervals of 1hr and on six different days for intra and inter day study, respectively. The Relative Standard Deviation (% RSD) was found to be less than 2. The results were shown in Table 2.

Table 2: Results of Precision study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg ml⁻¹)</th>
<th>Intraday precision % *RSD</th>
<th>Interday precision % *RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>20</td>
<td>0.319</td>
<td>0.112</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>15</td>
<td>0.416</td>
<td>0.209</td>
</tr>
<tr>
<td>Caffeine</td>
<td>12</td>
<td>0.511</td>
<td>0.336</td>
</tr>
</tbody>
</table>

* mean of six observations
LOD and LOQ
Limit of detection and limit of quantitation was estimated using formula 3.3 $\sigma$/S and 10 $\sigma$/S, respectively, where $\sigma$ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot. The results of sensitivity study is represented in Table 3.

Table 3: Summary of LOD & LOQ.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ibuprofen</th>
<th>Paracetamol</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.5437 μg/ml</td>
<td>0.641 μg/ml</td>
<td>0.195 μg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.986 μg/ml</td>
<td>2.671 μg/ml</td>
<td>0.825 μg/ml</td>
</tr>
</tbody>
</table>

Accuracy
To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by the standard addition method. The recovery studies were carried out at three different levels i.e. 80%, 100% and 120% level. The percentage recovery values were shown in Table 3.

Table 3: Accuracy

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Recovery</th>
<th>% *RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>99.69</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>0.197</td>
<td>0.346</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100.37</td>
<td>99.86</td>
</tr>
<tr>
<td></td>
<td>0.612</td>
<td>0.513</td>
</tr>
<tr>
<td>Caffeine</td>
<td>99.33</td>
<td>99.80</td>
</tr>
<tr>
<td></td>
<td>0.225</td>
<td>0.434</td>
</tr>
</tbody>
</table>

* mean of six observations

Analysis of formulation
The total amount of IBF, PCM and CFN present in formulation was calculated using the simultaneous equation. The obtained spectrum of sample (marketed formulation) is shown in Figure 8. The results were shown in Table 4.

Table 4: Analysis of formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim</th>
<th>Amount estimated</th>
<th>% Label claim</th>
<th>% *RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>400</td>
<td>398.46</td>
<td>100.03</td>
<td>0.498</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>325</td>
<td>323.45</td>
<td>99.52</td>
<td>0.476</td>
</tr>
<tr>
<td>Caffeine</td>
<td>25</td>
<td>24.98</td>
<td>99.95</td>
<td>0.474</td>
</tr>
</tbody>
</table>

* mean of six observations
CONCLUSION

The developed UV spectrophotometric method is simple, precise, accurate and cost effective for the estimation of IBF, PCM and CFN in pharmaceutical dosage forms. Drugs can be estimated without any interference from the excipients. It can be successfully applied for the routine analysis of all the three drugs in pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Principal, Modern college of pharmacy, Pune, India for providing necessary infrastructural facilities. Thanks are also extended to Emcure Pharmaceuticals, Pune, India for providing gift samples of the pure drugs for research work.

REFERENCES


