INTRODUCTION

Paracetamol (acetaminophen), N-(4-Hydroxyphenyl)acetamide (Fig.1) is a widely used analgesic and antipyretic agent for the relief of fever, headaches, minor pains, etc. It is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs and opioid analgesics, Paracetamol is used also in the management of severe pain (such as post operative pain). Paracetamol alone or in combination with other drugs is reported to be estimated by titrometry [1-2], spectrophotometric method [3-5], HPLC [6-7], TLC [8], HPTLC [9], UHPLC [10], LC-MS [11], FT-IR [12], amperometric determination [13] and fluorimetry [14].

Fig. 1: Structure of Paracetamol

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Fig. 2: Structure of Codeine Phosphate

In an effort to develop a simple and accurate method for routine analysis, this study describes HPLC-UV method, for the simultaneous determination of these drugs. Validation of the current method is performed according to the requirements of International Conference on Harmonisation (ICH) guideline [29].

MATERIALS AND METHODS

Materials and Chemicals

Paracetamol and Codeine phosphate reference standards were obtained from Sigma Aldrich. Tablet formulation containing Codeine phosphate hemihydrates 30 mg and Paracetamol 500 mg were obtained commercially. HPLC grade Methanol was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation and Chromatographic Conditions

HPLC analysis was performed by isocratic elution with a flow rate 1.0 ml/min. A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD quaternary pump) with an auto sampler, Shimadzu DDU-20A® vacuum degasser and a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software. Separation was carried out at 30°C using LiChroPrep® RP-18 (250 x 4.6 mm) column packed with octadecylsilil silica gel 5 µm. The detector was set at 210 nm. The chromatogram is shown in Fig. 3.
Preparation of Buffer Solution
2.04 g of monobasic potassium phosphate were dissolved into 1000 ml of water, and adjusted pH to 2.5 with o-phosphoric acid.

Preparation of Standard Solution
500 mg of Paracetamol and 30 mg Codeine phosphate working standards (accurately weighed) were transferred into a 100 ml volumetric flask. After addition of about 50 ml mobile phase, the mixture was sonicated for about 10 min and after made up to the volume. The stock solution was suitably diluted to produce a concentration of 0.5 mg/ml of Paracetamol and 0.03 mg/ml of Codeine phosphate respectively.

Sample Preparation
Twenty tablets were weighed, finely powdered and the average weight was determined. A portion of powder equivalent to 500 mg Paracetamol and 30 mg Codeine phosphate was transferred into a 100 ml volumetric flask and 50 ml of mobile phase was added and sonicated for 10 minutes to effect complete dissolution of both substances. The suspension was then made up to volume with mobile phase and after filtered. The aliquot portion of the filtrate was further diluted to get final concentration of 500 μg/ml of Paracetamol and 30 μg/ml of Codeine phosphate. 20 μl of the test solution were injected, chromatogram was recorded and the amounts of the drugs were calculated.

RESULTS AND DISCUSSION
The developed method for determination of Paracetamol and Codeine phosphate was further validated by using the following parameters:

Selectivity
Selectivity of the current method was demonstrated by good separation of the two active ingredients (Paracetamol and Codeine phosphate). Furthermore, matrix components, e.g. excipients, do not interfere with the two analytes.

Linearity
Standard solutions containing Paracetamol (100-1000 µg/ml) and Codeine phosphate (6-60 µg/ml) were prepared in the mobile phase. Triplicate 20 µl injections were made for each standard solution to estimate the reproducibility of the detector response at each concentration level and chromatographed under the conditions described above. The area of each peak was plotted against the concentration to obtain the calibration graph (Fig.4 and 5). The five concentrations of each compound were subjected to regression analysis to calculate the calibration equation and correlation coefficients.

The results obtained are shown in the Tables 1-2 and show that the current method was linear for the two analytes in the range specified above with a correlation coefficients better than 0.999.

Table 1: Linearity data for Paracetamol

<table>
<thead>
<tr>
<th>Linearity Level</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>194811</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>397621</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>795244</td>
</tr>
<tr>
<td>4</td>
<td>750</td>
<td>1192864</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>1590483</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td></td>
<td>0.999</td>
</tr>
</tbody>
</table>

Table 2: Linearity data for Codeine phosphate

<table>
<thead>
<tr>
<th>Linearity Level</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>10977</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>23953</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>47906</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>71859</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>95812</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td></td>
<td>0.999</td>
</tr>
</tbody>
</table>

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
LOD and LOQ were experimentally verified by six injections of Paracetamol and Codeine phosphate at the appropriate concentrations. The LOD was calculated to be 1.0 and 0.06 µg/ml and the LOQ was calculated to be 10.0 and 0.6 µg/ml for Paracetamol and Codeine phosphate, respectively.

Precision
The system precision of this method was evaluated by calculating the %RSD of the peak areas of six replicate injections of the standard solution, which were found to be 0.39% and 0.37%. For method precision evaluated with six sample replicate injections were found to be 0.41% and 0.39% for Paracetamol and Codeine phosphate respectively and it was found to be less than 1.0% shown in the Table 3.

Table 3: Results of precision for Paracetamol and Codeine phosphate

<table>
<thead>
<tr>
<th></th>
<th>Paracetamol</th>
<th>Codeine phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>System Precision %RSD</td>
<td>0.39</td>
<td>0.37</td>
</tr>
<tr>
<td>Method Precision %RSD</td>
<td>0.41</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Accuracy
Accuracy of the method was calculated by recovery studies. It is carried out by preparing the samples of 50%, 100% and 150% of target concentration. The samples were prepared in triplicate in each level. The results of studies along with its evaluation are given in the Table 4.
Table 4: Results of % recovery studies for Paracetamol and Codeine phosphate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery</th>
<th>Amount present</th>
<th>Amount recovered</th>
<th>% recovered</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine phosphate</td>
<td>50%</td>
<td>15 mg</td>
<td>14.80</td>
<td>99.66</td>
<td>1.728</td>
<td>1.731</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>30 mg</td>
<td>29.80</td>
<td>99.33</td>
<td>1.099</td>
<td>1.102</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>50%</td>
<td>45 mg</td>
<td>45.13</td>
<td>100.3</td>
<td>0.868</td>
<td>0.865</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>250 mg</td>
<td>249.7</td>
<td>99.88</td>
<td>1.022</td>
<td>1.023</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>500 mg</td>
<td>501.1</td>
<td>100.2</td>
<td>0.543</td>
<td>0.544</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>750 mg</td>
<td>748.4</td>
<td>99.79</td>
<td>1.031</td>
<td>1.029</td>
</tr>
</tbody>
</table>

CONCLUSION

An accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Paracetamol and Codeine phosphate was developed and validated for quality control analysis in combined tablets. The proposed method is rapid, where the total analytical run time for both drugs are less than 8 min and shows high degree of accuracy and precision with less than 2 % RSD. It is convenient for laboratory quality control of tablet dosage forms containing both substances.

REFERENCES