Abstract
This RP-HPLC method has been described for the determination binary formulated tablets of paracetamol and caffeine. However, none have been cross validated using formulated products. The present study depicts an accurate, stable, rapid, simple, precise and reducible reversed-phase high performance liquid chromatographic (RP-HPLC) separation method. The present isocratic method was carried out on C₁₈ column with mobile phase methanol and water in the ratio of 40:60 (by volume) at the flow rate 1.0 mL/minute. The detection was carried out at λ_max=243 nm. The retention time of paracetamol and caffeine were 3.03 and 4.23 minutes, respectively. Under the optimal condition, the linearity were found for paracetamol R²=0.999 and for caffeine R²=0.994. The limit of detection and limit of quantification for paracetamol were computed 0.04 and 0.12 µg/mL and for caffeine 0.05 and 0.15 µg/mL, respectively. The recoveries of paracetamol and caffeine were in the range of 99.62-99.45 % and 104.48-100.56 %. The proposed method was successfully validated to determine paracetamol and caffeine from its formulated tablets.

Key words: Development, Paracetamol, Caffeine, RP-HPLC, Validation

1. INTRODUCTION
Paracetamol is used as antipyretic, analgesic and anti-inflammatory. Chemically, it is 4-Hydroxy Acetanilide. This is available in combination form in different formulations. The antipyretic, analgesic and anti-inflammatory effect of paracetamol is due to inhibiting prostaglandin synthesis cyclooxygenase-1(COX-1) and cyclooxygenase-2 (COX-2) [1-3]. It is prevent to fever, headaches, pain of arthritis, aches, colds, flu and period pain. Paracetamol in combination form with caffeine used in migraine attack, child birth and avoid postpartum hemorrhage [4]. Caffeine is the most wanted drug of the world [5]. The chemical name of caffeine is 1, 3, 7-trimethylxanthine. Caffeine is used as a stimulant, due to acts on the central nervous system [6]. Paracetamol in formulated combination form with caffeine and other drugs has been validated various methods with spectrophotometry and high performance liquid chromatography [7-14]. The main aim of this study was to develop a new RP-HPLC method for determination of paracetamol and caffeine from formulated tablets, in accordance with International conference harmonized guidelines [15].

2. EXPERIMENTAL
2.1 Chemicals and Reagents
Paracetamol and caffeine reference standard (label claim 99.7%pure) was purchased from Ranbaxy Pharmaceutical Ltd. Tablets of paracetamol and caffeine with 250 mg and 100 mg purchased from Lupin Pharmaceutical Ltd. HPLC grade methanol and water were purchased from Merck India Limited and 0.45µm nylon membrane filter was supplied by Pall life Sciences, Mumbai.

2.2 Instrumentation
The instrument, a reversed phase-high performance liquid chromatograph was used for method development. The chromatograph consisting, a binary pump, column thermostat and a UV-detector. The eluent was analyzed using a C₁₈ column at wavelength (λ_max) 243 nm with mobile phase methanol and water in the ratio of 40:60 (by volume). Injection volume was used 20 µL. The mobile phase was filtered through 0.45µm nylon filter membrane followed by sonicate for five min prior to use.

2.3 Preparation of Stock Solution
Stock solution containing 250 µg/mL of paracetamol and 100 µg/mL of caffeine were prepared separately in mobile phase. The stock solutions were filtered through a 0.45 µm nylon membrane followed by sonicate for five minutes. Serial dilutions were prepared by appropriate dilution of the stock solutions with mobile phase.

2.4 Methods Validation
The methods were validated according to ICH guidelines.
with respect to linearity, specificity, precision, and system suitability test, limit of detection (LOD) and limit of quantification (LOQ) [15].

2.5 Linearity

For the measuring linearity were prepared serial dilutions of 5, 10, 20, 25, 50 and 100 µg/mL from the stock solution of paracetamol and caffeine. A total volume of 10 mL was maintained with mobile phase methanol and water. These different serial dilutions were filtered through a 0.45µm nylon membrane and sonicate. Each solution of 20 µL was injected into the column thrice. The calibration curves were obtained by plotting peak areas versus known concentrations in µg/mL.

2.6 Specificity

Specificity was determined with exipient of formulated tablets. An equivalent weight was taken and solution prepared similarly to the sample solution. The prepared solution was determined as per the described method. After determination was not reported any interference with exipient at the retention time of the peaks of paracetamol and caffeine. Therefore, it is concluded that the method is specific.

2.7 Accuracy

The accuracy of the method was determined by recovery method. The recovery was checked at the five theoretical concentrations levels 10, 20, 40, 50 and 100 µg. The chromatograms were recorded and the percentage recovery was calculated.

2.8 System Suitability test

The Reproducibility of sample was checked of the system to measurement of peak area and was carried out using three replicates of same concentration of standard and sample, respectively.

2.9 Limit of Detection (LOD) and limit of Quantification (LOQ)

It is a lowest response of the instrument at lowest concentration, so the detectable signal value called as limit of detection and quantifiable noise value called as limit of quantification. It was measured by signal to noise ratio. To determine the limit of detection (LOD) and limit of quantification (LOQ), to prepare three replications [16-19] of low concentrations serial dilutions of mixed standard of paracetamol and caffeine from the standard stock solution.

3. Results and Discussion

3.1 Selection of mobile phase

It was a basic need of liquid chromatography. To select the mobile phase various composition of mobile phases were checked with water and methanol in different ratio 25:75, 35:65 and 45:55 (by volume) on C¹ eight column at wave length 243 nm and methanol and water 20:80, 30:70 and 40:60 (by volume) on C¹ eight column at wave length 243 nm. The mobile phase methanol and water in the ratio of 40:60 (by volume) was selected. At this mobile phase was obtained suitable retention time and peak area of both drugs with better resolution.

3.2 Chromatographic Conditions

Under optimal experimental conditions, a mobile phase methanol and water in the ratio of 40:60 (by volume), and a flow rate of 1.0 mL/min, paracetamol and caffeine depicted a well defined chromatographic separation (resolution factor more than 1.423±0.07) within a run time of 6 min. The retention times of paracetamol and caffeine 3.03 minutes±0.36 and 4.23 minutes±0.39, respectively. Figure 1 depicts chromatograms of paracetamol and caffeine in binary mixture.

3.3 System suitability test

To establish the chromatographic conditions were performed system suitability test (SST) during the development and optimization of the method. The test was performed by injecting the standard mixture in triplicate and the various parameters retention time, tailing factor, resolution factor and theoretical plates were computed as reported by USP [20] and International conference harmonized guidelines. System suitability parameters were shown in table 1.

3.4 Linearity

Linearity was determined by the regression analysis. The calibration curves were plotted between known concentrations and average peak areas. The method was linear with a correlation co-efficient (R²) 0.999 and caffeine having correlation co-efficient (R²) 0.994. The result was shown in table 2.

3.5 Limits of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the signal-to-noise-ratio of 3 and 10. The limit of detection (LOD) and limit of quantification (LOQ) values were found for paracetamol 0.04 and 0.12 µg/mL and for caffeine 0.05 and 0.15 µg/mL, respectively.

3.6 Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs (figure 1).

3.7 Accuracy

The accuracy of the method was computed by determination of recovery for five concentrations. The amount of paracetamol and caffeine recovered and then percentage of drug content calculated. The mean recovery
The above method can be recommended for simultaneous determination of paracetamol and caffeine. Hence, the results were statistically significant. The recovery results showed that the method was very accurate for quantitative determination of paracetamol and caffeine from formulated tablets.

### 4. Conclusion

The RP-HPLC validation method was developed with isocratic mode. Selected experimental methods were providing high resolution and repeatability of peaks. For the repeatability of the peaks and retention time the required temperature was 20°C [21]. This validated method more reliable to simultaneous determination of paracetamol and caffeine from its binary formulated tablets. There was no interference from the excipients used in the tablet formulations and hence the methods are suitable for analysis of formulated tablets. The results of validation show that the described HPLC reverse phase separation methods are simple, linear, precise, accurate and selective. Hence the above method can be recommended for simultaneous determination of paracetamol and caffeine.

### References

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4. www.healthcaremagic.com
10. Tsvetkova, B; Pencheva, I; Zlatkov, A; Peikov, P;

### Tab. 1. Statistical Summary of validation system suitability parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Paracetamol</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>3.03 minutes ± 0.36</td>
<td>4.23 minutes ± 0.39</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.82 ± 0.06</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>Resolution factor</td>
<td>1.42 ± 0.07</td>
<td>1.33 ± 0.06</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>475.34 ± 0.44</td>
<td>2012 ± 0.05</td>
</tr>
</tbody>
</table>

SE= Standard Error (±)

### Tab. 2. Statistical summary of Linearity, limit of detection and limit of quantification.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Paracetamol</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples per curve</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Correlation range (µg/mL)</td>
<td>10-100</td>
<td>10-100</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y=4629x+34584</td>
<td>Y=860.4x-1056</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>R²=0.999</td>
<td>R²=0.994</td>
</tr>
<tr>
<td>Limit of detection (µg/mL)</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Limit of quantification(µg/mL)</td>
<td>0.12</td>
<td>0.15</td>
</tr>
</tbody>
</table>

### Tab. 3. Recovery experiment for paracetamol and caffeine

<table>
<thead>
<tr>
<th>Added in µg</th>
<th>Paracetamol</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found</td>
<td>Recovery %</td>
<td>Added in µg</td>
</tr>
<tr>
<td>10</td>
<td>9.96</td>
<td>99.62</td>
</tr>
<tr>
<td>20</td>
<td>19.14</td>
<td>95.72</td>
</tr>
<tr>
<td>40</td>
<td>39.9</td>
<td>99.96</td>
</tr>
<tr>
<td>50</td>
<td>51.4</td>
<td>102.89</td>
</tr>
<tr>
<td>100</td>
<td>99.45</td>
<td>99.45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paracetamol</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean=99.53</td>
<td>Mean=100.98</td>
</tr>
<tr>
<td>SD=2.55</td>
<td>SD=3.79</td>
</tr>
<tr>
<td>CV%=2.56</td>
<td>CV%=3.75</td>
</tr>
</tbody>
</table>

SD=standard deviation
CV% = coefficient variation percentage

of paracetamol and caffeine were found 99.53 % and 100.98 % with less than 10 % coefficient variation (table 3) [7]. Hence, the results were statistically significant. The recovery results showed that the method was very accurate for quantitative determination of paracetamol and caffeine from formulated tablets.

International Journal of Chromatographic Science 2013, 3(2): 31-34


Source of support: Nil;
Conflict of interest: The authors declare no conflict of interest.