High Performance Liquid Chromatographic Assay method for the determination of Paracetamol and Caffeine in Tablet Formulation-in vitro dissolution studies

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ABSTRACT

A simple high performance liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Paracetamol and Caffeine in combination. The mobile phase was prepared by mixing solvents, Methanol Buffer (70:30) v/v ratio. The Buffer consists of equal volume of 0.01 (M) ortho Phosphoric Acid and 0.01 (M) Monobasic Sodium Phosphate, pH adjusted to (4.5±0.2) with orthophosphoric acid. The column used was Separation Zorbax, (150x4.6mm) Ÿμm C-18 Column with flow rate of 1 ml / min using PDA detection at 254 nm. Ambroxol (50 g/ml) was used as internal standard. The retention times of Paracetamol and Caffeine, Ambroxol were found to be 3.2, 5.26 and 8.11 min respectively. The results of the study showed that the proposed HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Paracetamol and Caffeine, Ambroxol bulk drug and in its pharmaceutical dosage form.

Key words: HPLC, Paracetamol and Caffeine, Ambroxol, Dissolution.

INTRODUCTION

Paracetamol (4-acetaminophenol) is one of the most common drugs used in the world. It causes reduction in the amount of prostaglandin, therefore, helps to prevent headache and other pain like migraine headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, general pain, toothache, teething pain, period pain, and also used for the reduction of fever of bacterial or viral origin. It is suitable for most people, including elderly and young children, because it has very few side effects. Literature survey revealed the most recent methods for determination of paracetamol like chromatographic 1-4, electrochemical 5-6 and spectrophotometric 7-9 techniques. Caffeine (1, 3, 7 trimethylxanthine) is mainly used as diuretic, stimulant to the central nervous and to the cardiovascular systems 10. The use of the mixture of paracetamol and caffeine as an analgesic and antipyretic is well established in pharmaceutical formulation 11. Caffeine is estimated using Spectrophotometry 12, HPLC 13-14 and FTIR 15-16. Reverse phase High performance liquid Chromatography 17, have been described in literature for the determination of paracetamol and caffeine in various biological and pharmaceutical preparations. Author of the article and his research team has developed a HPLC method development in different pharmaceutical dosage form 17-20. The objective of the present investigation was to establish and validate the fast and sensitive high performance liquid chromatography (HPLC) method for dissolution studies, simultaneous determination of paracetamol, and Caffeine in tablets and internal standard Ambroxol.

Materials and Methods

Chemicals and solvents
Sodium Phosphate Monobasic, Methanol, Ortho-Phosphoric Acid, MilliQ water. All chemicals and solvents used were of GR/HPLC grade of Rankem, India Limited. Multi component tablet formulation of Paracetamol (500 mg) and caffeine (32 mg) were considered for analysis. Analytical reagent grade chemicals were used throughout the experiment.

HPLC System
The HPLC system consisted of a solvent delivery module Agilent 1100 Series Isocratic pump equipped with 20 μl loop and G1365B Multi Wavelength Detector. Integration was achieved by using the software Chemstation. Separation was carried out on a Zorbax, (150x4.6mm) Ÿμm C-18 Column.

Chromatographic Condition
The mobile phase was prepared by mixing solvents, Methanol Buffer (70:30) v/v ratio. The Buffer consists of equal volume of 0.01 (M) ortho Phosphoric Acid and 0.01 (M) Monobasic Sodium Phosphate, pH adjusted to (2.5±0.2) with orthophosphoric acid. The prepared mobile phase was filtered through a Millipore 0.45 μm membrane filter and ultrasonically degassed prior to use. Methanol and Water in the ratio of 70:30 (v/v) was used as diluents throughout the experiment. The detection wavelength was set at 254 nm. The elution was done at a flow rate of 1.0 ml/min under ambient condition.

Preparation of stock, working standard solutions, and sample solution
A stock solution of Paracetamol and Caffeine (100 μg/mL) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-ml volumetric flasks. They were dissolved in 25 mL of mobile phase and then the volume was made up to the mark to get 100 μg/mL. The internal standard solution was prepared by taking 10 mg of Ambroxol in a 100 ml standard flask. It is dissolved by adding 25 ml of mobile phase, shaken for few minutes to get a clear solution and the final volume was made up to 100 ml. For each drug, appropriate aliquots were pipette out from the standard stock solution into a series of 10-ml volumetric flasks. For each drug, appropriate aliquots were pipette out from the standard stock solution into a series of 10 ml volumetric flasks to get a concentration of 15, 30, 60, 90, 120 and 150 g/mL of Paracetamol, 5,10,20,30,40 and 50 g/mL Caffeine and 50 g/mL of Ambroxol (Internal Standard).

Dissolution study
For the dissolution study11-12 of Paracetamol and Caffeine analysis was done by using above chromatographic conditions. For this study standard solution of Paracetamol and Caffeine was prepared in dissolution media. For sample preparation an intact tablet was dissolved in 0.1 N HCL media (RPM 100). Sample was collected in dissolution vials after 2 hrs and then decanted the 0.1 N HCL media and the 5.0 pH phosphate buffer media was loaded and set RPM 100. Samples were collected in dissolution vials after different time intervals and filtered through 0.45 μm filter. Equal volumes (50 μL) of these solutions were injected into the chromatograph by auto sampler and peak areas were measured.

Effect of pH
The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 3.0-6.0. Shows that a pH of 5.0 was the most appropriate one giving well-resolved peaks and highest no. of theoretical plates.

Assay
Twenty tablets were finely powdered and weighed accurately in the electronic balance. The powder equivalent to 500 mg of paracetamol, and 32 mg of Caffeine were weighed accurately and dissolved in 250 ml methanol (HPLC Grade). The solution was filtered through 0.45 μm filter and degassed prior to use. A stock solution of Paracetamol and Caffeine (100 μg/mL) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-ml volumetric flasks. They were dissolved in 25 mL of mobile phase and then the volume was made up to the mark to get 100 μg/mL. The internal standard solution was prepared by taking 10 mg of Ambroxol in a 100 ml standard flask. It is dissolved by adding 25 ml of mobile phase, shaken for few minutes to get a clear solution and the final volume was made up to 100 ml. For each drug, appropriate aliquots were pipette out from the standard stock solution into a series of 10-ml volumetric flasks. For each drug, appropriate aliquots were pipette out from the standard stock solution into a series of 10 ml volumetric flasks to get a concentration of 15, 30, 60, 90, 120 and 150 g/mL of Paracetamol, 5,10,20,30,40 and 50 g/mL Caffeine and 50 g/mL of Ambroxol (Internal Standard).

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Drug | Concentration of Std Solution used (µg/mL) | Concentration of Sample Solution Added (µg/mL) | Amount Found (µg/mL) | % Recovery | % RSD
---|---|---|---|---|---
Paracetamol | 40 | 50 | 53.65 | 100.22 | 0.876
| 40 | 45 | 48.87 | 101.65 | 0.654
| 40 | 35 | 40.41 | 100.97 | 0.235
Caffeine | 5 | 20 | 26.77 | 99.98 | 0.652
| 5 | 15 | 19.04 | 99.06 | 0.188
| 5 | 10 | 13.54 | 100.63 | 0.558

Table 3: System suitability parameters

<table>
<thead>
<tr>
<th>Property</th>
<th>Paracetamol</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>k'</td>
<td>5.26</td>
<td>2.12</td>
</tr>
<tr>
<td>t</td>
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<td>1.21</td>
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<tr>
<td>S</td>
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<td>11432</td>
</tr>
<tr>
<td>R</td>
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<td>5.33</td>
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</table>

Analysis of formulation

Twenty tablets of Paracetamol and Caffeine in combination were weighed, their average weight was determined and finally they were crushed to a fine powder. The tablet powder equivalent to 15 mg of Paracetamol and 45 mg of Caffeine was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase, and then the volume was made up to the mark with the mobile phase. The content was ultrasonicated for 30 min for complete dissolution. The solution was then whirled filter paper No-41. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 40 µg/mL of Paracetamol and 5 µg/mL of Caffeine, which was falling in the Beer’s-Lamberts range with 50 µg/mL internal standard, showed good results and was selected for the entire analysis. The results of tablet analysis (n = 6) were found to be 99.86% with ±0.25% standard deviation (SD) and 99.62% with ±0.36% SD for Paracetamol, and Caffeine respectively. From the typical chromatogram of Paracetamol, Caffeine and Ambroxol (internal standard), it was found that the retention time of Paracetamol was 2.3 min, Caffeine was 4.1 min and Ambroxol was 5.1 min, which were well-resolved peaks with a resolution factor of 7.8 and 8.3. The results analysis was shown in Table 2.

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations 80, 100, and 120% of those expected, in accordance with ICH guidelines, by replicate analysis (n = 6). Standard drug solutions were added to a pre analyzed sample solution and percentage drug content was measured. From these results it was clear that the method enables very accurate quantitative estimation of Paracetamol, and Caffeine in dosage form, because all the results were within acceptable limits, i.e. COV < 2.0% and S.D. < 1.0.

Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of all the two drugs. It was observed that three sharp peaks for Paracetamol, and Caffeine were obtained at retention times 5.26 and 8.11 min respectively; these peaks were not obtained from placebo solution. The specificity of the method was assessed by comparing chromatogram obtained from drug standards with that obtained from capsules solutions. The retention times of the standards were well-resolved peaks with a resolution factor of 7.3 and 8.3. The results analysis was shown in Table 2.

CONCLUSION

New, Stability indicating HPLC method has been developed for simultaneous analysis of Paracetamol, and Caffeine in formulation. It was shown above that the method was accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The run time is relatively short, which enables rapid quantitation of many samples in routine and quality control analysis of capsule formulations. The same solvent was used throughout the experimental work and no interference from any excipients was observed. These results show the method could find practical application as a quality-control tool for simultaneous analysis of two drugs from their combined dosage forms in quality-control laboratories.

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REFERENCES


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