Comparative *In vitro* Assessment of Quality Control Parameters of Omeprazole Enteric-Coated Pellets in Capsules from Local Pharmacies in Bangladesh

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Research Article

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**Additional Declarations:** The authors declare potential competing interests as follows: I declared that there is no conflict of interest to disclose this article.
Abstract

Backgrounds:
Omeprazole as enteric coated pellets in capsules form is used for the therapy of gastric and duodenal ulcer. This product is now manufacturing all over Bangladesh for the healthcare of gastric patient. The main objective of this study is to evaluate the quality of Omeprazole Capsules as enteric coated pellets form through ensuring chemical test like assay, dissolution and impurity from 5 pharmaceutical companies from local pharmacies in Bangladesh.

Methods

The enumerated HPLC (high performance liquid chromatography) methodology of British Pharmacopoeia followed for the metage of assay, impurity and dissolution of Omeprazole Capsules as enteric coated pellets form.

Results

The assay, impurity and dissolution results of all brands of Omeprazole Capsules as enteric coated pellets form met the specification limit specified in the British Pharmacopeia. Assay results from all brands were between 96.12% ± 1.24 to 101.12% ± 1.01. In case of product release, all the products are treated with pH 4.5 (phosphate buffer) for 45 minutes found from 1.41% ± 2.04 to 7.12% ± 1.24 and the same stressed product treated for pH 6.8 (phosphate buffer) for 45 minutes and found 84.02% ± 0.24 to 97.23% ± 0.84. Omeprazole Impurity C and D are the known identified impurity of Omeprazole Capsules. The content of Omeprazole impurity D was from 0.32% ± 0.21 to 0.12% ± 0.39 and total impurity found from 0.45% ± 0.11 to 0.28% ± 0.09. Omeprazole impurity C was not detected in sample solution. As the results, all the brands sample meet the specification limit of specified monograph so it can be concluded that in terms of quality, integrity and efficacy, pharmaceutical market manufacturing Omeprazole capsules in Bangladesh maintain the highest standard for providing appropriate therapy.

Conclusion

The results are found satisfactory for both the lower- and top-class pharmaceutical companies in Bangladesh also ensuring the high quality standard for Omeprazole Capsules. This report will help the drug control authority to gather information about the existing quality status of medicine for Omeprazole Capsules as enteric coated pellets form available in different pharmacies of Bangladesh.

INTRODUCTION
Proton pump inhibitors (PPIs) are a family of medications that inhibits or remove the production of gastric acid acting on the proton pump present in gastric parietal cells. It interact with H+/K+-ATPase, the proton pump, reversibly reducing or blocking its activity. This influences the final step of the gastric acid secretion process and acts irrespectively both in the basal acid secretion and in the one instigated by stimuli. PPIs are quickly active and with a unique daily dose can maintain control of the inhibition of the gastric acid secretion\textsuperscript{1}. Being unstable in acidic pH, Omeprazole is marketed as enteric-coated pellets encased in hard gelatin capsules. Thus, post-marketing follow-up is necessary to monitor probable changes which may affect the performance of Omeprazole capsules\textsuperscript{2}. Omeprazole seems to be well absorbed from the gastrointestinal tract (GIT). However, its oral bioavailability in humans is about 40 to 50%, suggesting that the drug goes through a pronounced first-pass metabolism before entering the systemic circulation\textsuperscript{3}. So, we can develop the drug delivery system to reduce the first pass metabolism. Omeprazole is widely used for the treatment of GERD (gastro-oesopagal reflux disease) and lesion\textsuperscript{4}. World Health Organization Proclaimed to all manufacturing drug product company for ensuring proper product quality and safety\textsuperscript{5}.

About 853 as Omeprazole generic product is now available in Bangladesh\textsuperscript{6}. Approximately 300 pharmaceutical companies are manufacturing pharmaceutical product in Bangladesh. The needs of medicine of Bangladeshi people are fulfilled only 3% by importing them whereas the 97% demands are ensured by the local manufacturing pharmaceutical companies\textsuperscript{7}. Bangladesh exports now pharmaceutical products to 151 countries, including those in the EU, Africa and Latin America as well as the United States of America\textsuperscript{8}. If these sectors can improve more, it might be the first exporting sector in Bangladesh. One of the major obstacles for manufacturing pharmaceuticals is importing raw material. However, a few of pharmaceutical companies has initiated for manufacturing active ingredient in Bangladesh\textsuperscript{9}.

This research will create awareness to physician, drug control authority and they can audit to the pharmaceutical companies so that they are bound to manufacture the pharmaceutical products with high quality standard. This study will also provide knowledge about the assay, impurity and dissolution of pharmaceutical product available in Bangladesh and specification of them specified in pharmacopeia.

**MATERIALS AND METHODS**

Five pharmaceutical companies have chosen based on top, middle and low class in Bangladesh. For each of company bought 6 different batches of Omeprazole capsules of same strength 20 mg from different district of Bangladesh (Dhaka, Gajipur, Rajshahi, Narayanganj & Naogaon) from the pharmacy dispenser. Specified British Pharmacopeia method for Omeprazole Capsules was used for the evaluation of them. In order to perform in vitro test validated method was used.

**Study Design**
Sample Collection and Identification

Based on top, middle and bottom of pharmaceutical companies in Bangladesh 5 pharmaceuticals are chosen\textsuperscript{10}. The physical appearance of sample, manufacturing date, expiry date, DAR number, manufacturer name were properly checked during the time of purchase. After that the samples were coded as OC-1, OC-2, OC-3, OC-4 and OC-5 for proper analysis. Validated method should be used for the quantification of the market product\textsuperscript{11}. Accuracy, precision, specificity, robustness are some common parameters that should be considered for the method validation\textsuperscript{12}.

Instruments

Laboratory instruments such as Dissolution tester with auto sampler (Electro lab, India), High Performance Liquid Chromatography with PDA detector (Waters Alliance, USA), Analytical Balance (Sartorius, Switzerland), Semi micro-Balance (Metler Toledo, Switzerland) were used for this study.

Chemical Reagents and Standard

Potassium dihydrogen phosphate (Sharlau, Spain), Disodium hydrogen phosphate (Merck, Germany), Sodium dihydrogen phosphate (Sharlau, Spain), Sodium hydroxide pellets (Merck, Germany), Acetonitrile (Sharlau, Spain), pH meter (Metler Toledo, Switzerland), Distilled water (Ultrapure water) were used. All the reagents were used analytical grade. The reference standard of Omeprazole was obtained from a local pharmaceuticals as a gift sample for research. The purity of Omeprazole reference standard was 99.53% on as is basis

In Vitro Quality Control Test

Drug Assay Test\textsuperscript{13}

Preparation of Buffer Solution: Dissolved 1.4012 g of disodium hydrogen orthophosphate into 1000 mL of purified water. Adjust pH 7.61 with orthophosphoric acid.

Preparation of Mobile Phase: Taken 270 mL of acetonitrile and 730 mL of buffer solution to make 1000 mL of mobile phase. Filtered through 0.2µm membrane filter and degassed by sonication for about 5 minutes prior to use.

Chromatographic Condition: HPLC column (4.0-mm × 25-cm; 4-µm packing USP L7), injection volume (40µL), wavelength (305nm), flow rate (1.0 mL/min) were attributed as instrument parameter for operation.

Preparation of Standard Solution: Taken 24.12 mg Omeprazole BP standard into 200 mL volumetric flask. Added 150 mL of mobile phase and sonicated for 10 minutes with intermittent shaking. Cooled to room temperature. Diluted up to the mark with mobile phase. Taken 5.0 mL of the solution into 50 mL volumetric flask. Diluted up to the mark with mobile phase. Concentration: 0.012 mg/mL.
Preparation of Sample Solution: Weighed 20 capsules and transferred the contents into a mortar. Weighed the empty capsule shell and crush the contents (pellets) of 20 capsules. Transferred the contents 106.23 mg of Omeprazole BP into 200 mL volumetric flask. Added 150 mL of mobile phase and sonicated for 12 minutes with intermittent shaking. Cooled to room temperature. Diluted up to the with mobile phase. Centrifuged at 5000 rpm at 5 minutes. Taken 5.0 mL of the solution into 50 mL volumetric flask. Diluted up to the mark with mobile phase.

Impurity Test

Preparation of Buffer Solution: Dissolved 1.4120 g of disodium hydrogen orthophosphate into 1000 mL of purified water. Adjust pH 7.62 with orthophosphoric acid

Preparation of Mobile Phase: Taken 270 mL of acetonitrile and 730 mL of buffer solution to make 1000 mL of mobile phase. Filtered through 0.2µm membrane filter and degassed by sonication for about 5 minutes prior to use.

Chromatographic Condition: HPLC column (4.0-mm × 25-cm; 4-µm packing USP L7), injection volume (40µL), wavelength (280nm), flow rate (1.0 mL/min) were attributed as instrument parameter for operation.

Preparation of Solution-1: Taken 106 mg Omeprazole BP crushed pellets into 200 mL volumetric flask. Add 150 mL of mobile phase and sonicated for 12 minutes with intermittent shaking. Cooled to room temperature. Diluted up to the with mobile phase. Centrifuged 5000 rpm for 5 minutes. Taken the supernatant solution for HPLC vial.

Solution-2: Taken 5.0 mL of sample solution into 100 mL volumetric flask. Diluted up to the mark with mobile phase. Taken 1.0 mL of the solution into 10 mL volumetric flask. Diluted up to the mark with mobile phase.

Preparation of system suitability solution:

Omeprazole Stock Solution: Taken 10.12 mg of Omeprazole BP into 10 mL volumetric flask. Diluted up to the mark with mobile phase.

Omeprazole Impurity D: Taken 5.1342 mg of Omeprazole impurity D into 10 mL volumetric flask. Diluted up to the mark with mobile phase.

System suitability solution: Taken 2.0 mL of Omeprazole stock solution and 4.0 mL of Omeprazole impurity D stock solution into 20 mL volumetric flask with mobile phase.

Concentration: Omeprazole: 0.1 mg/mL; Omeprazole impurity D: 0.1 mg/mL

Dissolution Test
0.25M Trisodium hydrogen Phosphate: Dissolved 8.2121 g of trisodium hydrogen phosphate into 200 mL of water.

1M Sodium dihydrogen phosphate: Dissolved 6.2341 g of sodium dihydrogen phosphate into 50 mL of water.

0.5M Disodium hydrogen phosphate: Dissolved 35.5234 g of disodium hydrogen phosphate into 500 mL of water.

Solution C: Mixed 5.2 mL of 1M sodium dihydrogen phosphate and 63.2 mL of 0.5M disodium hydrogen phosphate and diluted with water to make 1000 mL. Adjusted pH 7.60 by orthophosphoric acid.

Preparation of Mobile Phase: Mixed 250 mL of solution C, 350 mL of water and 400 mL of acetonitrile to make 1000 mL of mobile phase. Adjust pH 7.6 if necessary. Filter through 0.2 µm membrane filter and degas by sonication for about 5 minutes prior to use.

Preparation of Solution-A: Mixed 110 mL of 0.25M trisodium hydrogen phosphate, 220 mL of 0.5M disodium hydrogen phosphate and diluted with water to make 1000 mL. Adjusted pH to 11.0 by diluted orthophosphoric acid.

Dissolution medium pH 4.5: Dissolved 204.8121 g of potassium dihydrogen phosphate into 30000 mL of water. Adjusted pH to 4.52 by diluted orthophosphoric acid

10M sodium hydroxide: Dissolved 30 g of sodium hydroxide pellets into 75 mL of water.

Solution B: Mixed 75 mL of 10M sodium hydroxide and 7425 mL of 0.05 M pH 4.5

Dissolution Condition
<table>
<thead>
<tr>
<th>Parameters</th>
<th>First Stage at pH 4.5</th>
<th>Second Stage at pH 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>pH 4.5</td>
<td>pH 6.8</td>
</tr>
<tr>
<td>Volume</td>
<td>700 mL</td>
<td>900 mL (700mL 1&lt;sup&gt;ST&lt;/sup&gt; stage + 200 mL Solution B)</td>
</tr>
<tr>
<td>Apparatus</td>
<td>Paddle with Sinker</td>
<td>Paddle with Sinker</td>
</tr>
<tr>
<td>RPM</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Temperature</td>
<td>OC-1: 36.55, 36.59, 37.11, 37.25, 37.44, 37.14</td>
<td>OC-2: 36.75, 36.69, 37.14, 37.15, 37.04, 37.12</td>
</tr>
<tr>
<td></td>
<td>OC-3: 36.55, 36.62, 37.11, 37.12, 37.14, 37.41</td>
<td>OC-4: 36.65, 36.61, 37.01, 37.11, 37.12, 37.41</td>
</tr>
<tr>
<td></td>
<td>OC-5: 36.82, 36.63, 37.21, 37.21, 37.32, 37.22</td>
<td>OC-5: 36.82, 36.63, 37.21, 37.21, 37.32, 37.22</td>
</tr>
<tr>
<td>Run time</td>
<td>45 minutes</td>
<td>10, 15, 30 &amp; 45 minutes</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>10 mL after 45 minutes</td>
<td>10 mL</td>
</tr>
<tr>
<td>Replacement</td>
<td>10 mL of pH 4.5</td>
<td>10 mL of pH 6.8</td>
</tr>
</tbody>
</table>

Preparation of Sample Solution at pH 4.5 stage: Taken 5.0 mL of sample solution into 25 mL volumetric flask. Diluted up to the mark with solution A.

Preparation of Sample Solution at pH 6.8 stage: Taken 5.0 mL of sample solution into 25 mL volumetric flask. Diluted up to the mark with solution A.

Preparation of Standard Solution at pH 4.5 stage: Taken 20.12 mg of Omeprazole BP into 100 mL volumetric flask and dissolved in 50 mL of solution A. Diluted up to the mark with water. Taken 5.0 mL of the solution into 50 mL volumetric flask. Diluted up to the mark with 50% solution A. Taken 5.0 mL of the solution into 25 mL volumetric flask. Diluted up to the mark with 50% solution A. Taken 3.0 mL of the solution into 20 mL volumetric flask. Diluted up to the mark with 50% solution A.

Concentration: 0.0006 mg/mL

Preparation of Standard Solution at pH 6.8 stage: Taken 20.45 mg of Omeprazole BP into 100 mL volumetric flask and dissolved in 50 mL of solution A. Diluted up to the mark with water. Taken 5.0 mL of the solution into 50 mL volumetric flask. Diluted up to the mark with 50% solution A. Taken 5.0 mL of the solution into 25 mL volumetric flask. Diluted up to the mark with 50% solution A.
Concentration: 0.004 mg/mL

Chromatographic Condition: HPLC column (3.0-mm × 15-cm; 5-µm packing USP L1), injection volume (10µL), wavelength (302nm), flow rate (0.25 mL/min), column temperature (30°C) were attributed as instrument parameter for operation.

**Statistical Analysis**

% The highest assay value for the Omeprazole Capsule found 101.12% for OC-2 manufacturer company and on the other hand the lowest assay value was 96.12% for OC-4 manufacturer. However, all of the manufacturer assay results were met the limit. Similarly, the maximum total impurity and Omeprazole impurity D content was found more than 0.45% and 0.30% for OC-5 manufacturer. The lowest total impurity and Omeprazole impurity D was found about 0.30% for OC-3 and about 0.15% for OC-2. The content of Omeprazole D in OC-5 was found almost double relative to the impurity in OC-2.

Dissolution Data Evaluation

At pH 4.5 stages minimum release found around 1% for OC-5 brand and maximum release found for around 7% for OC-3. All of markets samples meet the specification limit. This sample again treated with pH 6.8 to observe to release at buffer stage. Here, the maximum release found at 97% for OC-4 on the contrary the lowest release at 45 minutes observed for 84% for OC-1. However, all of the market samples show the similar type release at buffer stage and they meet the specification limit.

**RESULTS**

Table-1: Evaluation of Assay of 5 Pharmaceutical Products in Market

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OC-1</th>
<th>OC-2</th>
<th>OC-3</th>
<th>OC-4</th>
<th>OC-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>97.12</td>
<td>101.12</td>
<td>100.12</td>
<td>96.12</td>
<td>98.34</td>
</tr>
<tr>
<td>SD</td>
<td>0.21</td>
<td>0.78</td>
<td>1.51</td>
<td>0.94</td>
<td>0.56</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.26</td>
<td>1.01</td>
<td>1.97</td>
<td>1.24</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Table-2: Evaluation of % Impurity Content

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OC-1</th>
<th>OC-2</th>
<th>OC-3</th>
<th>OC-4</th>
<th>OC-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole Impurity D</td>
<td>0.21</td>
<td>0.15</td>
<td>0.17</td>
<td>0.19</td>
<td>0.34</td>
</tr>
<tr>
<td>Any other Impurity</td>
<td>0.14</td>
<td>0.11</td>
<td>0.17</td>
<td>0.12</td>
<td>0.19</td>
</tr>
<tr>
<td>Total Impurity</td>
<td>0.41</td>
<td>0.32</td>
<td>0.29</td>
<td>0.36</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table-3: Evaluation of % release of drug at buffer stage
**DISCUSSION**

The purpose of this study was to evaluate quality of Omeprazole Capsules available in pharmacies of Bangladeshi market. For this reason 5 manufacturing companies were chosen based on their rank and collected the samples from the local pharmacies of Bangladesh for the chemical test for ensuring quality product. Assay, dissolution and impurity are the most critical attributes that can ensure a product quality. So, the samples were tested by HPLC as per British Pharmacopoeia standard and found satisfactory results for all of the manufacturing company of Bangladesh. This is a positive fact for the Bangladeshi patient and also for the manufacturing companies. The physician and drug control authority will get meaning information about the status of Omeprazole Capsules in Bangladeshi market.

However, this research has been conducted with a few samples from few of pharmaceutical company. If the number of pharmaceutical companies for sample collection is high then the quality information for Omeprazole Capsule in Bangladesh will be much more precise to present and informative. Moreover, robustness is the integral part of method validation. So, the method should use the robustness to capture its capacity for pharmaceutical product quantification\textsuperscript{17}.

**CONCLUSION**

In order ensure and evaluate the quality of Omeprazole Capsules which are now available in Bangladesh Market, 5 pharmaceutical companies medicines were purchased by myself from different district of Bangladesh and they were conducted for laboratory test. The results are found satisfactory for all of the selected pharmaceutical companies. The data also reported that the lower class pharmaceutical company in Bangladesh also ensuring the high quality standard for Omeprazole Capsules. This study can help the drug control authority to get an idea about the existing quality status of medicine for Omeprazole Capsules as enteric coated pellets form.

**Abbreviations**

BP
British Pharmacopoeia
mL
CONFLICT OF INTEREST

There is no conflict of interest to disclose this article.

FUNDING

The research was done from self-fund. The reagents, sample and standards were purchased from the local retailers of Bangladesh.

References


**Figures**
Figure 1

% Assay value

Figure 2

% Assay value
**Impurity Profile**

### Figure 3

**% Dissolution at Acid**

![Graph showing % Dissolution at Acid](image)

### At buffer stage dissolution

![Graph showing % Release vs Time in min](image)
Figure 4

Dissolution at Buffer

Figure 5

Unnumbered image in the Results section.

Specimen Chromatogram for assay test (HPLC Chromatogram)

Figure 6

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time (min)</th>
<th>Angle</th>
<th>Purity %</th>
<th>Purity Threshold</th>
<th>ID</th>
<th>Name</th>
<th>ID</th>
<th>Match</th>
<th>Match ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimcrelize</td>
<td>3.44</td>
<td>30.3</td>
<td>7.05</td>
<td>0.41</td>
<td>342</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Cimcrelize</td>
<td>7.628</td>
<td>9.783</td>
<td>0.41</td>
<td>0.41</td>
<td>302</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Cimcrelize</td>
<td>6.824</td>
<td>6.824</td>
<td>1.754</td>
<td>1.754</td>
<td>302</td>
<td>1.75</td>
<td>1.75</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>Cimcrelize</td>
<td>11.420</td>
<td>6.16</td>
<td>1.039</td>
<td>1.039</td>
<td>302</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Cimcrelize</td>
<td>52.678</td>
<td>0.227</td>
<td>1.256</td>
<td>1.256</td>
<td>302</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Unnumbered image in the Results section.

**Specimen Chromatogram for impurity test (HPLC Chromatogram)**

![Chromatogram](image)

**Figure 7**

Unnumbered image in the Results section.

**Specimen Chromatogram for dissolution test (HPLC Chromatogram)**