Determination of Omeprazole in Bulk and Injectable Preparations by Liquid Chromatography

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An accurate, simple, reproducible, and sensitive liquid chromatographic method was developed and validated for the determination of omeprazole in powder for injection and in pellets. The analyses were performed at room temperature on a reversed-phase C18 column of 250 × 4.6 mm id, 5 μm particle size. The mobile phase, composed of methanol–water (90 + 10, v/v), was pumped at a constant flow rate of 1.5 mL/min. Detection was performed on a UV detector at 301 nm. The method was validated in terms of linearity, precision, accuracy, and ruggedness. The response was linear in the range 32–48 μg/mL ($r^2 = 0.9976$). The relative standard deviation values for intra- and interday precision studies were 1.22 and 1.56% for injectable and 2.13 and 2.45% for pellets, respectively. Recoveries ranged between 95.81 and 100.48%.

Omeprazole (OMP), a substituted benzimidazole, is an irreversible inhibitor of gastric hydrogen-potassium adenosine triphosphate pump, which is the final common step of acid secretion in parietal cells. It is used to treat gastric reflux esophagitis and the Zollinger-Ellison syndrome (1). OMP is a weak base ($pK_a = 4.2$ and $pK_a = 9$) freely soluble in ethanol, methanol, and methylene chloride. It is slightly soluble in acetone and isopropanol, very slightly soluble in water, and dissolves in dilute solutions of alkali hydroxides (2). The stability of OMP is a function of pH; it is rapidly degraded in acid media, but has acceptable stability under alkaline conditions. OMP is available in capsules and powder for injection and paste formulations. Several liquid chromatography (LC) methods have been reported for the determination of OMP and its metabolites in various biological fluids (3–14). Analytical methods described in the literature for determination of OMP in pure form or pharmaceutical formulation include thin-layer chromatography (15), differential pulse polarographic (16), and spectrophotometry (17–20). Titrimetry (2) and LC (21) are official methods for determination of OMP in bulk material. LC has been used for determination of OMP in pharmaceutical formulations. A stability-indicating LC method for quantitation of OMP in capsules has been reported (22), and LC with coulometric detection was applied to the determination of OMP in a paste formulation (23). The LC methods described above use a buffer in mobile phase or coulometric detection. For routine quality control, the development of a simple, rapid, and sensitive method is highly desirable. LC assays for determination of OMP in powder for injection and pellets have not been reported. The present paper describes a rapid, simple, and precise LC method for determination of OMP in these pharmaceutical formulations.

Experimental

Reagents and Standard

(a) OMP.—Reference standard (U.S. Pharmacopeia, Rockville, MD); the injectable drug was obtained commercially.
(b) OMP pellet (10%).—Galena (São Paulo, Brazil).
(c) Methanol.—LC grade (Tedia Co. Inc., Fairfield, OH).
(d) Sodium hydroxide and sulfuric acid.—Merck (Darmstadt, Germany). Solvents were filtered through a 0.45 μm membrane and degassed.
(e) Ultra-pure water.—Obtained from a Labconco water purification unit (Kansas City, MO).

Chromatographic Conditions and Instrumentation

The LC system consisted of a Shimadzu (Kyoto, Japan) LC-10 A equipped with a Model LC-10 ADVP pump, an SPD-10 A VP UV-Vis detector, an SCL-10 A VP system controller, SIL-10 A VP autoinjector, and a degasser module; data were acquired and processed by Shimadzu class-VP 5.0 software. A 250 × 4.6 mm id stainless steel LiChrospher® 100 column prepacked with 5 μm RP-18 end-capped (Merck) guard cartridge system was a LiChroCart 4-4 RP-18. The mobile phase consisted of methanol–water (90 + 10, v/v). The flow rate was 1.5 mL/min. Detection was performed at 301 nm. The LC system was operated at room temperature. The injection volume was 20 μg/mL for all standards and samples.

Sample Preparation

(a) Injectable.—The contents of 5 ampules of lyophilized OMP were diluted with 0.1N NaOH and transferred to 500 mL volumetric flask for a final concentration of...
0.4 mg/mL. This stock solution was diluted to final concentration of 0.4 μg/mL with methanol.

(b) Pellets.—An amount equivalent to 40 mg OMP was weighed and transferred to a 100 mL volumetric flask with 0.1N NaOH. A 5 mL amount of this solution was diluted with methanol in a 50 mL volumetric flask (40 μg/mL).

Method Validation

(a) Linearity.—A stock solution of 0.4 mg/mL was prepared in a 50 mL volumetric flask by dissolving 20 mg OMP reference standard USP in methanol. Appropriate amounts of the stock solutions were diluted with methanol, yielding concentrations of 32.0, 36.0, 40.0, 44.0, and 48.0 μg/mL. Triplet injections of each were made.

(b) Limits of detection (LOD) and quantitation (LOQ).—The LOD and LOQ were calculated by using the calibration line directly. The aforementioned factors (3.3 and 10) were multiplied by the ratio from the residual standard deviation and the slope (corresponding to the standard error of the slope).

(c) Accuracy.—Accuracy was evaluated by fortifying an OMP sample solution (0.4 mg/mL) with 3 known concentrations of reference standard (6.0, 8.0, and 12.0 μg/mL). The recovery of added drug was determined.

(d) Precision.—Repeatability was calculated by assaying 6 samples of the 100% standard concentration (40.0 μg/mL). Intermediate precision was assessed by comparing the results obtained from 6 samples prepared by 2 different analysts on 2 different days.

(e) System suitability test.—Relative standard deviations (RSDs) of the area, tailing factor, and retention time were the chromatographic parameters selected for the system suitability test.

(f) Specificity.—Forced degradation studies were performed to evaluate the specificity of the method. Degraded samples were prepared by subjecting 1.0 mg/mL reference standard solution to acid (1N sulfuric acid) and base (0.1N NaOH) until boiling (ca 1 min). The samples were allowed to cool at room temperature and neutralized (if needed). Oxidative condition was obtained by mixing the reference standard solution (1.0 mg/mL) with 30% (v/v) hydrogen peroxide at room temperature for 1 h. All samples were diluted to 40 μg/mL with methanol.

(g) Ruggedness.—Ruggedness was established by changing the chromatographic system (column, flow rate, mobile phase, and wavelength).

Results and Discussion

The described reversed-phase LC method was developed to provide a rapid quality control determination of OMP in powder for injection and in pellets. This method uses a simple mobile phase and is considered more useful than LC methods cited in the literature. The physico-chemical properties of OMP, such as solubility, polarity, and UV absorption, influenced the chromatographic conditions. The mobile phase was chosen after several trials with methanol and water. The optimum mobile phase was methanol–water (90 + 10, v/v); flow rate, 1.5 mL/min; detection wavelength, 301 nm; retention time, 1.77 min.

![Figure 1. Chromatograms of OMP at 40 μg/mL.](image)

(A) Reference substance, (B) powder for injection, and (C) pellets. Chromatographic conditions: column, C18 (250 × 4.6 mm id, 5 μm particle size); mobile phase, methanol–water (90 + 10, v/v); flow rate, 1.5 mL/min; detection wavelength, 301 nm; retention time, 1.77 min.

The UV spectrum of OMP shows an intense absorption at 301 nm and this wavelength was chosen for the analyses. No interference from the sample excipients could be observed at this detection wavelength.

Validation of the method was performed according to the International Conference on Harmonization (24). Linearity of the detector responses was determined by preparing calibrations graphs. The linearity of the peak area responses versus concentration was studied from 32 to 48 μg/mL (corresponding from 80 to 120% of the test concentration). The representative linear equation for OMP was:

\[ y = 45,589x - 224,393 \]
where $x$ is concentration in $\mu g/mL$. The LOQ and LOD calculated were 7.25 and 2.39 $\mu g/mL$, respectively.

The experimental values obtained for the determination of OMP in samples are presented in Table 1. The precision and accuracy of the assay were demonstrated. The precision is usually expressed as the RSD of a series of measurements. The interday precision of the assay showed good results: 98.48%, RSD 1.22% for powder and 99.40%, RSD 2.13% for pellets. The intraday precision was performed by assaying the samples on 2 different days by 2 different analysts and showed RSD values of 1.56 and 2.45% for powder and pellets, respectively. The accuracy was evaluated by fortifying the samples with OMP standard at 3 levels and assaying by the proposed procedure. Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. The mean recovery was 98.77%. The RSDs of the symmetry and the peak area response were <2%. The retention time was 1.77 ± 0.044 min (RSD < 1%). The method exhibited good ruggedness.

It was confirmed that changes of chromatography conditions (wavelength, columns, flow rate, analysts) did not influence the analytical results. The results of forced degradation showed no interference with the OMP peak. As reported in the literature (22), OMP was stable under basic conditions. The drug decomposed rapidly in acid, and mild degradation was observed under oxidative condition. Degradation peaks were observed in these conditions; however, resolution of the OMP peak from the other peaks was <2%. The purity of the OMP peak was not investigated and the specificity of the method was not demonstrated conclusively on the basis of the results for acid and oxidative stress.

### Table 1. Data obtained for validation of OMP in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Calibration range</th>
<th>Equation</th>
<th>$r^2$</th>
<th>LOD</th>
<th>LOQ</th>
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<tbody>
<tr>
<td>32–48 $\mu g/mL$</td>
<td>$y = 45589x - 224393$</td>
<td>0.9976</td>
<td>2.39 $\mu g/mL$</td>
<td>7.25 $\mu g/mL$</td>
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</table>

### Accuracy (% of recovery) for powder

<table>
<thead>
<tr>
<th>Spiked concentration, $\mu g/mL$</th>
<th>Measured concentration, $\mu g/mL$</th>
<th>Recovery, %</th>
<th>Mean recovery, %</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>6.03</td>
<td>100.48</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.00</td>
<td>100.02</td>
<td>98.77</td>
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<tr>
<td>12</td>
<td>11.50</td>
<td>95.81</td>
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### Precision (repeatability)

<table>
<thead>
<tr>
<th>Sample (n = 6)</th>
<th>Theoretical amount</th>
<th>Experimental amount, mg ± SD</th>
<th>Purity, %</th>
<th>RSD, %</th>
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</thead>
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<tr>
<td>Powder</td>
<td>40 mg</td>
<td>39.38 ± 0.48</td>
<td>98.44 ± 1.20</td>
<td>1.22</td>
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<tr>
<td>Pellets</td>
<td>10%</td>
<td>9.940 ± 0.212</td>
<td>99.90 ± 2.11</td>
<td>2.13</td>
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</tbody>
</table>

### Conclusions

The LC method developed in this study has the advantage of simplicity, precision, accuracy, and convenience. The method uses simple reagents, with minimal sample preparation procedures. The results demonstrated that this method is useful for the routine quality control of OMP in powder for injection and pellets. Because of the short analysis time, it can be used in process control of this drug.

### References


