Simultaneous Determination of Omeprazole, Tinidazole and Clarithromycin in Bulk Powder and Helicure® Tablets by HPLC

Hesham Salem¹, Safa M Riad², Mamdouh R Rezk³, Kholoud Ahmed¹*¹

¹Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, Egypt
²Analytical Chemistry Department, Faculty of Pharmacy-Cairo University, Kasr El-Aini Street, Egypt

Abstract

Sensitive and precise chromatographic method was developed and validated for simultaneous determination of omeprazole (OMP), tinidazole (TND) and clarithromycin (CLR) in bulk powder, laboratory prepared mixture and pharmaceutical preparation. The technique adopted for quantification is HPLC. A mixture of acetonitrile, methanol, phosphate buffer at pH 3.5 (33: 17: 50, v/v/v) was used as a mobile phase. The stationary phase used was (150 mm×4.6 mm, 10µm) C8 Lichrosorb™ analytical column. The method was linear in the range of 0.2-250 µg mL⁻¹, 0.5-250 µg mL⁻¹ and 75-2000 µg mL⁻¹ for OMP, TND and CLR respectively. The selectivity of the proposed method was checked using laboratory prepared mixtures. The proposed method was successfully applied to the analysis of OMP, TND and CLR in their mixture and in pharmaceutical dosage form without interference from other additives.

Keywords: Omeprazole; Tinidazole; Clarithromycin; HPLC.

Introduction

Omeprazole (OMP), is 6-methoxy-2-[[4-(methoxy-3,5-dimethyl-2-pyridinyl) methyl sulphinyl]-1H-benzimidazole [1], (Figure 1). It is the first member of the "proton pump inhibitors" that are widely used for the prophylaxis and treatment of both gastrointestinal ulcers and symptomatic gastro-esophageal reflux. It is highly effective in the treatment of Zollinger-Ellison syndrome [2]. Tinidazole (TND) is 1-[2-(ethyl sulphonyl) ethyl]-2-methyl-5-nitro-1H-imidazole, [1] (Figure 2). It is used as antiprotozoal agent. Clarithromycin (CLR), is (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-a-L-ribohexopyranosyl) oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[(3,4,6-trideoxy-3-(dimethylamino)-b-D-xly hexopyranosyl) oxy] oxacyclotetradecane-2,10-dione (6-O-methylerythromycin A), [1] (Figure 3). CLR is semi-synthetic macrolide antibacterial agent [1].

The literature survey reveals several analytical methods for quantitative estimation of OMP alone in body fluids and in pharmaceutical formulations these methods include spectrophotometry [3-14], electrochemical methods [15], HPLC [16-21], liquid chromatography-electrospray ionization tandem mass spectrometry [22] and electrophoresis [23]. Tinidazole was estimated in body fluids and in pharmaceutical formulations by spectrophotometry [24-29], potentiometry [29], HPLC methods [29-31], polarography [32,33] and resonance light scattering technique [34]. Clarithromycin has been reported to be estimated in body fluids and in pharmaceutical formulations by spectrophotometry [35], HPLC methods [36-44]. Omeprazole, Tinidazole and Clarithromycin were simultaneously determined by spectrophotometry [45,46].

Up to our knowledge, there is no isocratic HPLC method was described for the simultaneous determination of the three studied drugs in their laboratory prepared mixtures and in the pharmaceutical dosage form without prior derivatisation. The present work aimed to develop an isocratic HPLC method for simultaneous determination of OMP, TND and CLR in laboratory prepared mixtures and pharmaceutical dosage form. The proposed method has advantage of being cheap, simple, rapid and time saving (one run in less than 7 minutes).

Experimental

Instruments

A liquid chromatogram consisted of an quaternary pump (Agilent model G1316 A/G1316 B), a diode array multiple wavelength detector (model G1316 C/D and G1365C/D, Agilent 1200 Series), Standard and preparation autosamplers (Agilent 1200 series) equipped vacuum degasser, Agilent. Stationary phase (150 mm×4.6 mm, 10 µm)
C8 Lichrosorb™ analytical column. Mobile phase: acetonitrile, methanol, buffer at pH 3.5 (33:17:50, v/v/v). The mobile phase was filtered through a 0.45 µm Millipore membrane filter and was degassed for 15 min in an ultrasonic bath prior to use. UV-detection was done at 210 nm. The samples were filtered also through a 0.45 µm membrane filter. To reach good equilibrium, the analysis was usually performed after passing 50-60 mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. The relative peak area ratios were then plotted versus the corresponding concentrations of OMP, TND and CLR to get the calibration graphs and to compute the corresponding regression equations.

Standards, solvents, and pharmaceutical preparation

Reference omeprazole (OMP), reference tinidazole (TND) and reference clarithromycin (CLR) were kindly donated by EGYPHAR Pharmaceuticals Co. The potency was found to be 100.30%, 100.13% and 100.16% for OMP, TND and CLR, respectively. Pharmaceutical dosage form (Heli-cure tablets) were kindly supplied by EGYPHAR and were claimed to contain 20 mg of OMP, 500 mg TND and 250 mg of CLR per tablet. Acetonitrile, methanol (HPLC grade) and phosphate buffer adjusted to pH 3.5.

Standard solutions

OMP, TND standard solutions (each 0.5 mg mL⁻¹) and CLR standard solution (2 mg mL⁻¹) were prepared in mobile phase for the suggested HPLC method. The standards solutions were freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 hr.

Procedures

Linearity: Portions of OMP, TND standard solutions (each 0.5 mg mL⁻¹) and CLR standard solution (2 mg mL⁻¹) were transferred separately into a series of 10-mL volumetric flasks and completed with mobile phase. Several dilutions were done and the content of each flask was completed to volume with the mobile phase to get the concentrations of 0.2-250 µg mL⁻¹ OMP, 0.5-250 µg mL⁻¹ TND and 75-2000 µg mL⁻¹ CLR. The samples were then chromatographed using the following chromatographic condition. Stationary phase (150 mm×4.6 mm,10 µm) C8 lichrosorb™. Many mobile phases such as methanol and acetonitrile (50:50, 60:40, 65:35, by volume), methanol, acetonitrile and phosphate buffer adjusted at pH 3.5 (30:20:50, 40:30:30) by volume and different other ratios but the mobile phase which give the best separation and peaks shape was found to be a mixture of acetonitrile, methanol, buffer at pH 3.5 (33:17:50, v/v/v). The mobile phase was filtered through a 0.45 µm millipore membrane filter and was degassed for about 15 min in an ultrasonic bath prior to use, flow rate; 0.7 mL min⁻¹ [isocratically at temperature (35°C)], with UV-detection at 210 nm, the detection wavelength was set regarding the UV absorption spectra of the drugs (Figure 5) and their relative concentrations within the pharmaceutical formulation. Whereas TNZ is nominally 2 and 25 times more concentrated than CLR and OMP, respectively. The drugs have strong contributions in the overall UV region (200-375 nm). This is why an optimum detection wavelength was set at 210 nm during the chromatographic separation, favoring the quantification of both CLR and OMP, which represent the less concentrated components of this ternary mixture. In addition, this chosen detection wavelength can greatly improve the sensitivity of the proposed method for the CLR determination because it exhibits absorption maxima (at 210 nm). The samples were filtered also through a 0.45 µm membrane filter. To reach good equilibrium, the analysis was usually performed after passing 50-60 mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. The relative peak area ratios were then plotted versus the corresponding concentrations of OMP, TND and CLR to get the calibration graphs and to compute the corresponding regression equations.

Analysis of laboratory prepared mixtures containing different ratios of OMP, TND and CLR: Aliquots of each standard solution were mixed to prepare different mixtures containing different ratios (3:4:90, 1:0.2:26, 7:4:130, 2:0.2:23, 0.5:50:336, 30:12.5:168, 6:2.5:124, 0.5:1:144) of OMP, TND and CLR, respectively. The drugs were calculated from the corresponding regression equations.

Assay of pharmaceutical formulations (Heli-cure tablets): Twenty tablets were powdered well and homogeneously mixed in a mortar. A mass of the powdered tablets equivalent to 20 mg of OMP, 250 mg of CLR and 500 mg of TND was weighed and transferred to a 100-mL volumetric flask. The powder was extracted by shaking with 3×30 mL mobile phase with vigorous shaking for 15 minutes then filtered. The volume was completed to the mark with the mobile phase. Several portions 0.5-2 mL of aliquot were transferred separately to 10-
mL volumetric flasks, the volumes were completed to the mark with mobile phase and chromatographed under the previous mentioned conditions.

### Results and Discussion

#### High-performance liquid chromatography

A simple isocratic high-performance liquid chromatographic method was developed for the determination of OMP, TND and CLR in bulk and in pharmaceutical preparation using (150 mm×4.6 mm, 10 µm) C8 lichrosorb™ analytical column. The mobile phase consisted of acetonitrile, methanol, buffer at pH 3.5 (33: 17: 50, v/v/v). The mobile phase was chosen after several trials to reach the optimum stationary/mobile-phase matching. The average retention times under the conditions described are 2.06 min for OMP, 1.36 min for TND and 5.44 for CLR (Figure 4). One sample can be chromatographed in less than 6 min.

Peak purity was confirmed for the HPLC peaks of OMP, TND and CLR by a pilot run using a photodiode array detector. Calibration graph was obtained by plotting the relative peak area ratios against concentrations. Linearity range was found to be 0.2-250 µg mL⁻¹ for OMP, 0.5-250 µg mL⁻¹ TND and 75-2000 µg mL⁻¹ CLR. The regression equation for OMP: \( A=0.1832C+0.1946 \) (\( r=0.9999 \)), for TND: \( A=0.0241C+0.0513 \) (\( r=0.9999 \)) and for CLR: \( A=0.0021C+0.0280 \) (\( r=0.9999 \)) where \( A \) is the relative peak area ratio, \( C \) is the concentration in µg mL⁻¹ and \( r \) is the correlation coefficient. The mean percentage recovery was found to be 100.08 ± 0.454 for OMP, 100.40 ± 0.535 for TND and 100.65 ± 0.862 for CLR (Tables 1 and 2).

#### Analysis of laboratory prepared mixtures containing different ratios of OMP, TND and CLR

The suggested HPLC method was successfully applied for the determination of the studied drugs in their laboratory prepared mixtures. The precision of the proposed method was checked by the analysis of different concentrations (Table 3). The mean percentage recovery was found to be 100.097 ± 0.216 for CLR. The suggested HPLC method was successfully applied for the determination of the studied drugs in their pharmaceutical formulation.

#### Analysis of dosage form (Heli-cure tablets)

The suggested HPLC method was successfully applied for the determination of OMP, TND and CLR in their pharmaceutical formulation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OMP</th>
<th>TND</th>
<th>CLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (µg mL⁻¹)</td>
<td>0.2-250</td>
<td>0.5-250</td>
<td>2000-75</td>
</tr>
<tr>
<td>Slope</td>
<td>0.183</td>
<td>0.024</td>
<td>0.002</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.195</td>
<td>0.051</td>
<td>0.028</td>
</tr>
<tr>
<td>Variance</td>
<td>0.206</td>
<td>0.286</td>
<td>0.743</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.454</td>
<td>0.535</td>
<td>0.862</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Accuracy mean RSD%</td>
<td>100.08</td>
<td>100.40</td>
<td>100.65</td>
</tr>
<tr>
<td>Precision (RSD%)</td>
<td>0.454</td>
<td>0.535</td>
<td>0.862</td>
</tr>
<tr>
<td>Repeatability</td>
<td>0.201</td>
<td>0.184</td>
<td>0.237</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>0.332</td>
<td>0.409</td>
<td>0.294</td>
</tr>
<tr>
<td>Specificity mean RSD%</td>
<td>99.96</td>
<td>100.14</td>
<td>99.97</td>
</tr>
<tr>
<td></td>
<td>0.407</td>
<td>0.332</td>
<td>0.216</td>
</tr>
</tbody>
</table>

Table 1: Validation and regression parameters for the determination of OMP, TND & CLR by the proposed HPLC method.

<table>
<thead>
<tr>
<th>Claimed amount taken (µg mL⁻¹)</th>
<th>Authentic added (µg mL⁻¹)</th>
<th>Authentic found (µg mL⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.00</td>
<td>20.00</td>
<td>20.11</td>
<td>100.55</td>
</tr>
<tr>
<td>50.00</td>
<td>40.11</td>
<td>100.28</td>
<td></td>
</tr>
<tr>
<td>60.00</td>
<td>60.14</td>
<td>100.23</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>100.35 ± 0.172</td>
</tr>
<tr>
<td>TND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.00</td>
<td>10.04</td>
<td>100.40</td>
<td></td>
</tr>
<tr>
<td>25.00</td>
<td>24.94</td>
<td>99.76</td>
<td></td>
</tr>
<tr>
<td>50.00</td>
<td>49.95</td>
<td>99.90</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>100.02 ± 0.336</td>
</tr>
<tr>
<td>CLR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000.00</td>
<td>1987.62</td>
<td>99.38</td>
<td></td>
</tr>
<tr>
<td>1500.00</td>
<td>1499.23</td>
<td>99.95</td>
<td></td>
</tr>
<tr>
<td>2000.00</td>
<td>3998.80</td>
<td>99.97</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>99.78 ± 0.335</td>
</tr>
</tbody>
</table>

Table 4: Application of the standard addition technique to the proposed HPLC method of OMP, TND & CLR in their pharmaceutical formulation.

Conclusion

Validation of the accuracy of the proposed HPLC method was...
The values in the parenthesis are corresponding theoretical t- and F-values at P=0.05 [44].

Table 5: Statistical comparison for the results obtained by the proposed method and the official method for analysis of OMP, TND and CLR in dosage form.

Table 7: System suitability parameters of the proposed HPLC method.

confirmed using standard addition technique (Table 4). Statistical comparison with the official and reported methods showed that the proposed HPLC is sensitive and precise (Table 5 and 6). Application of the proposed methods to the analysis of OMP, TND and CLR in their pharmaceutical formulation (Table 3) shows that excipients do not interfere with the determination. The system suitability parameters of the proposed HPLC method (Table 7). The proposed method has advantage of being sensitive and applicable over wide range. The proposed method can be used for routine analysis of omeprazole, tinidazole and clarithromycin in quality control laboratories.

References


