RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE MAGNESIUM AND DOMPERIDONE IN A TABLET DOSAGE FORM

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A simple, sensitive and validated isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of esomeprazole and domperidone in tablet dosage form. The chromatographic separation was achieved on a hyperchrome C-18 (4.6×150 mm, 5µ particle size) analytical column using a mixture of acetonitrile: phosphate buffer (pH 5.0) in the ratio of 60:40 (v/v) used as the mobile phase, at a flow rate of 1.0 ml/min and detector wavelength at 290 nm. The validation of the proposed method was carried out for specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. Linearity of method was found to be in concentration range 10-50 µg/ml for esomeprazole and 5-25 µg/ml for domperidone with correlation coefficient greater than 0.9999. The retention time of domperidone and esomeprazole was found to be 2.92 and 3.91 min respectively. The method is suitable for the estimation of both the components simultaneously in pharmaceutical tablet formulations.

Key words: Esomeprazole, Domperidone, RP-HPLC, Validation, Simultaneous estimation.

INTRODUCTION

Domperidone (DOM), which is chemically 5-chloro-1-[3-[2-oxo-2,3-dihydro-1H-benimidazol-1-yl]propyl]piperidin-4-yl]-1, 3-dihydro-2H-benimidazol-2-one (Figure 1), is used as an anti-emetic and to suppress nausea and vomiting.

Fig. 1. Chemical structure of Domperidone

DOM is indicated for treating symptoms associated with upper gastrointestinal motility disorders caused by chronic and sub-acute gastritis. It is a gastrointestinal emptying (delayed) adjuvant, a peristaltic stimulant and exhibits anti-emetic properties. It can be used in patients with Parkinson's disease (Shindler et al 1984) and also found to be effective in the treatment of gastroparesis (Silvers et al 1998). It is official in BP which recommends non-aqueous titration with perchloric acid as titrant and naphthol benzein as indicator (British Pharmacopoeia, 2009). Several chromatographic methods have been reported for determination of Domperidone in pharmaceutical dosage form by differential pulse voltammetry (El-Shahawi et al 2007), planar chromatography (Gosavi et al 2006), high-performance liquid chromatography
(Patel et al 2009; 2007; Thanikachalam et al 2008, Singh et al 2010), high-performance thin-layer chromatography (Patel et al 2008; Yadav et al 2009; Pawar et al 2010), UV-spectrophotometry (Kapil et al 2009; Rajendra Prasad et al 2009; Patel et al 2010; Zenita Devi et al 2012). For the determination of DOM in biological samples like human, dog and rat plasma, several chromatographic techniques such as liquid chromatography-mass spectrometry (Li et al 2009; Bose et al 2009), ultra performance liquid chromatography (Xu et al 2008) and high-performance liquid chromatography (Sivakumar et al 2008) have been reported. Esomeprazole magnesium trihydrate (Andersson et al 2001) (ESO) is chemically bis (5-methoxy-2-[(S)-[4-methoxy-3, 5-dimethyl-2-pyrindinyl] methyl sulfanyl]-1H-benzimidazol-1-yl) magnesium trihydrate, (Figure 2) a compound that inhibits gastric acid secretion. Esomeprazole is cost effective in the treatment of gastric oesophageal reflux diseases.

![Chemical structure of esomeprazole magnesium trihydrate](image)

**Fig. 2. Chemical structure of esomeprazole magnesium trihydrate**

Esomeprazole is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole (Scott et al 2002). Several methods have been employed for the estimation of esomeprazole alone and combination with other drugs such as UV and RP-HPLC method (Hultman et al 2007; Magesh et al 2010; Lakshmana Prabu et al 2008; Zanitti et al 2010; Jain et al 2011). Keeping in view the importance of RP-HPLC method for estimation of drugs (Prasanthi et al 2011; Bhimavarapu et al 2011; Basaveswara Rao et al 2012a; 2012b), the present work was directed toward the development of a new, rapid and sensitive RP-HPLC method for the simultaneous determination of ESO and DOM in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines (Code Q2A, Code Q2B) which are mandatory also.

**EXPERIMENTAL**

**Chemicals and Reagents**

Analytically pure sample of ESO was a generous gift from Glenmark Pharma Ltd., Baddi, and DOM was an obtained from Aurbindo Pharma Ltd, Hyderabad. Potassium dihydrogen phosphate, disodium hydrogen phosphate and acetonitrile (HPLC Grade) were purchased from E. Merck Ltd, Mumbai, India. The 0.45 µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd, Chandigad, India. Triple distilled water was used throughout the experiment. Commercial tablets of ESO and DOM, Ranidom-O (Mankind Pharma) was procured from the local drug market.

**Instrumentation**

Liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and photodiode array detector YL 9160 detector connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used. Weighing was done on Digital Micro Balance (CX-265) Citizen Scale (I) Pvt. Limited and pH of buffer was maintained by using Systronics pH meter.

**Chromatographic conditions**

The isocratic mobile phase consisted of acetonitrile: phosphate buffer (pH-5.0) in the ratio of 60:40 v/v, flowing through the column at a constant flow rate of 1 ml/min. A hyperchrome C-18 column (4.6 × 250 mm, 5 µ particle size) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 290 nm was selected as the detection wavelength for UV-PDA detector. The HPLC system was operated at room temperature 25°C.

**Standard preparation**

**Standard stock solution**

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of acetonitrile and phosphate buffer in the ratio of 60:40 (pH 5.0) to get concentration of 1000 µg/ ml.

**Working standard solution**

Working standard solutions were prepared by taking dilutions ranging from 10-50, 5-25 µg/ml for ESO and DOM respectively.
Sample preparation
The commercial formulations of DOM and ESO (Ranidom-O tab) were selected for analysis. Twenty tablets were weighed and powdered separately. Weight equivalent to 10 mg DOM and 20 mg ESO was dissolved in 100 ml diluents and then sonicated for 15 min and filtered through whatmann paper no. 41. Different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

Method validation
The developed chromatographic method was validated using ICH guidelines. Validation parameters performed include linearity, limit of detection and quantitation, selectivity, robustness, accuracy and repeatability as per ICH guidelines.

RESULTS AND DISCUSSION
In the present work, conditions were optimized for the development and validation of a simple and accurate HPLC method for the simultaneous determination of DOM and ESO in tablet dosage form. The most appropriate mobile phase composition was found to be mixture of acetonitrile: phosphate buffer (pH 5.0) in the ratio of 60:40 (v/v). Under the described experimental conditions, sharp peaks that belong to DOM and ESO were obtained at retention times of 2.92 and 3.91 min respectively as shown in Figure 2.

![Chromatogram for the analysis of DOM and ESO](image)

**Table 1. System suitability parameters**

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>ESO</th>
<th>DOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>3.91±0.0063</td>
<td>2.92±0.0064</td>
</tr>
<tr>
<td>No. of theoretical plate</td>
<td>4218±2.756</td>
<td>2718±1.549</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.26±0.012</td>
<td>1.43±0.013</td>
</tr>
<tr>
<td>HETP</td>
<td>0.059±3.87</td>
<td>0.091±5.24</td>
</tr>
<tr>
<td>Calibration range</td>
<td>10-50 µg/ml</td>
<td>5-25 µg/ml</td>
</tr>
</tbody>
</table>

*Each value is the Mean±SD of six determinations

**Linearity**
The calibration curve was linear over the concentration range of 5-25 µg/ml and 10-50 µg/ml for DOM and ESO respectively. The correlation coefficients in both cases were found to be greater than 0.9999 which manifested a linear relationship between concentration and the peak area. The linearity was represented by a linear regression equation as follows.

(ESO) \(Y = 30.098X + 1.876\) with correlation coefficient equal to 0.999.

(DOM) \(Y = 20.79X + 1.02\) with correlation coefficient equal to 0.9999.

**Accuracy**
Method accuracy was performed by adding known amounts of DOM and ESO to the pre-analyzed solution and then comparing the added concentrations with the found concentrations. Three levels of solutions were made which corresponded to 80, 100 and 120% of the nominal analytical concentration (10 µg/ml for ESO and 5 µg/ml for DOM). Each level was made in triplicate (Table 2). The mean percentage recoveries obtained for Domperidone and Esomeprazole magnesium trihydrate were 96.26 and 99.38% respectively and RSD was less than 2.
### Table 2. Results of recovery studies

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. of drug in preanalyzed samples (µg/ml)</th>
<th>Standard drug solution added (µg/ml)</th>
<th>Recovered amount* (µg/ml)</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESO</td>
<td>DOM</td>
<td>ESO</td>
<td>DOM</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>5</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of nine determinations (3 replicates at 3 concentration level)

#### Repeatability

Five dilutions in three replicates were analyzed in same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in Table 3.

#### Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD < 2) as shown in Table 3.

#### Robustness

As per ICH norms, small, but deliberate variation by altering the pH or concentration of the mobile phase were made to check the method’s capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of acetonitrile:phosphate buffer (pH 5.0) (60:40 v/v), acetonitrile: phosphate buffer (pH 5.0) (55:45 v/v), was used as a mobile phase. Results of analysis were summarized in Table 3.

### Table 3. Results of precision studies

<table>
<thead>
<tr>
<th>Validation Parameter</th>
<th>Percentage Mean±SD* (n=15)</th>
<th>Percentage RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESO</td>
<td>DOM</td>
</tr>
<tr>
<td><strong>Repeatability</strong></td>
<td>99.68±0.11</td>
<td>98.83±0.06</td>
</tr>
<tr>
<td><strong>Intermediate precision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day to Day</strong></td>
<td>99.92±0.11</td>
<td>98.80±0.05</td>
</tr>
<tr>
<td><strong>Analyst to Analyst</strong></td>
<td>99.93±0.13</td>
<td>98.85±0.05</td>
</tr>
<tr>
<td><strong>Robustness</strong></td>
<td>99.67±0.07</td>
<td>99.02±0.04</td>
</tr>
</tbody>
</table>

*Mean of thirty determinations (3 replicates at 5 concentration level)

### Table 4. Result of tablet analysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug</th>
<th>Mean*</th>
<th>SD*</th>
<th>% COV*</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ESO</td>
<td>99.37</td>
<td>0.0427</td>
<td>0.2300</td>
<td>0.0154</td>
</tr>
<tr>
<td>2</td>
<td>DOM</td>
<td>98.97</td>
<td>0.0514</td>
<td>0.4183</td>
<td>0.0184</td>
</tr>
</tbody>
</table>

*Mean of nine determinations (3 replicates at 3 concentration level)

### CONCLUSION

A simple, precise, reliable, rapid, sensitive and accurate reverse phase HPLC method has been developed for the simultaneous determination of esomeprazole and domperidone. The developed method is suitable for the identification and quantification of binary combination of esomeprazole and domperidone.
A high percentage of recovery and the run time of less than five min allowed its application for the routine determination of esomeprazole and domperidone in the pharmaceutical formulations.

REFERENCES


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