STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RABEPROAZOLE SODIUM AND MOSAPRIDE CITRATE IN BULK AND FORMULATION

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Objective: Development and validation of reversed phase liquid chromatographic method for the quantitative determination of Rabeprazole sodium and Mosapride citrate in bulk and combined dosage form.

Methods: A thermo Inert sil, C18 (250 x 4.6 mm i.d., 5 µ) column with mobile phase containing methanol:buffer (ammonium acetate pH 6.5):acetonitrile in the ratio of (50:20:30 %) was used. The flow rate was 1.0 ml/min, column temperature was 25 °C and effluents were monitored at 245 nm.

Results: The retention times of Rabeprazole sodium and Mosapride citrate were 2.951 min and 4.195 min, respectively. Correlation co-efficient for Rabeprazole sodium and Mosapride citrate was found to be 0.9999 and 0.9999, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Rabeprazole sodium and Mosapride citrate in formulations was found to be in the range of 97-103 % and 98-102 %, respectively confirms the non-interferences of the excipients in the formulation.

Conclusion: The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate in pharmaceutical dosage forms. Due to its simplicity, rapidness and high precision, the method was successfully applied to the estimation of Rabeprazole sodium and Mosapride citrate in combined dosage form.

Keywords: RP-HPLC, Rabeprazole sodium, Mosapride citrate, Methanol, Acetonitrile, Validation.

INTRODUCTION

Rabeprazole sodium is chemically (RS)-2-[(4-[3-methoxy propoxy]-3-methyl/propoxy-2-yl] methyl sulphonyl)-1H-benzo[d]imidazole (fig. 1). Rabeprazole sodium is an antulcer drug in the class of proton pump inhibitors. As an anti ulcer drug, it is used in short-term treatment in healing and symptomatic relief of duodenal ulcers and erosive or ulcerative gastro esophageal reflux disease (GORD); maintaining healing and reducing relapse rates of heartburn symptoms in patients with GORD; treatment of daytime and nighttime heartburn and other symptoms associated with GORD; long-term treatment of pathological hypersecretory conditions, including Zollinger-Ellison syndrome and in combination with Amoxicillin and Clarithromycin to eradicate Helicobacter pylori [1].

Mosapride citrate is chemically (RS)-4-amino-5-chloro-2-ethoxy-N-[(4-[4-fluorobenzyl] morpholin-2-yl)methyl]bezamide citrate (fig. 2). Mosapride is a gastro pro-kinetic agent that acts as a selective 5HT4 receptor agonist. The major active metabolite of Mosapride is known as M1, additionally acts as a SHT3 antagonist. In addition to its pro-kinetic properties, Mosapride also exerts anti-inflammatory effects on the gastrointestinal tract which may contribute to some of its therapeutic effects.

Mosapride also promotes neurogenesis in the gastrointestinal tract which may prove useful in certain bowel disorders. The neurogenesis is due to Mosapride’s effect on the 5-HT4 receptor where it acts as an agonist [2].

The drug analysis plays an important role in the development of drugs, manufacturing and therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final product obtained meets the required specification. The literature review indicates there are several analytical methods have been reported for estimation of these drugs as individual or in combination with other drugs, and also several analytical methods for the determination of simultaneous estimation of Rabeprazole sodium and Mosapride citrate by HPTLC, UPLC and UV in dosage formulation and its bioanalytical applications. Some of the reported RP-HPLC methods were not economical in terms of mobile phase composition, column dimensions and run times. Hence there is need for the development of newer method for estimation of Rabeprazole sodium and Mosapride citrate present in tablet to overcome above discussed hurdles. In addition, stability indicating RP-HPLC method for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate in pharmaceutical dosage form are very scanty. Hence the main objective of this study is to develop a stability indicating RP-HPLC method for estimation of Rabeprazole sodium and Mosapride citrate & validate the developed method according to ICH guidelines by using various parameters [3-15].

MATERIALS AND METHODS

Chemicals and reagents

Rabeprazole sodium and Mosapride citrate were obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Milli-Q water,
HPLC grade methanol, acetonitrile and analytical grade ammonium acetate buffer were purchased from E. Merck (India) Ltd, Mumbai.

**Instrumentation**

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and thermo Inert sil C18 (250 x 4.6 mm, i.d., 5 µ).

**HPLC conditions**

The mobile phase consisting of methanol, ammonium acetate buffer (pH 6.5) and acetonitrile (HPLC grade) were filtered through 0.45 µm membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 50:20:30 % into the column at a flow rate of 1.0 ml/min.

The column temperature was maintained as 25 °C. The detection was monitored at 245 nm and the run time was 8 min. The volume of injection loop was 10 µl prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

**Preparation of standard solution**

20 mg of Rabeprazole sodium and 15 mg of Mosapride citrate were accurately weighed and transferred into a 100 ml volumetric flask. Disolved with 25 ml of methanol and sonicated for 20 min.

Finally, the solution was diluted to the required volume using methanol. (200 µg/ml of Mosapride citrate and 150 µg/ml of Rabeprazole sodium). From the standard stock solution 2 ml was pipetted out into 10 ml volumetric flask and made up the volume with mobile phase. (40 µg/ml of Rabeprazole sodium and 30 µg/ml of Mosapride citrate).

**Preparation of sample solution**

Twenty capsules were accurately weighed and grinded to a fine powder. An amount of powder equivalent to 15 mg of Mosapride citrate and 20 mg of Rabeprazole sodium were weighed accurately and transferred into a 100 ml volumetric flask containing 25 ml of methanol and sonicated for 30 min and diluted to 100 ml with methanol, then the solution was filtered through 0.45 µm membrane filter and 2 ml of filtrate was taken into 10 ml volumetric flask and made up to the volume with methanol.

**Procedure**

10 µl of the filtered portion of the sample and standard preparations were injected into the chromatograph. The responses for the major peaks were recorded and the content of Rabeprazole sodium and Mosapride citrate was calculated.

**Validation parameters**

All the analytical validation parameters were determined according to ICH guidelines for the proposed analytical method [16-20]. The obtained validation parameters are presented in table 2.

**System suitability**

Standard solution was injected six times into system and chromatograms were recorded, % RSD (relative standard deviation) of retention time & peak area, theoretical plates and tailing factor were calculated.

**Accuracy**

Accuracy was determined in terms of % recovery. Sample solutions were prepared at three different concentration levels 50 %, 100 % and 150 %. Predetermined amount of standard was added to these solutions by spiking standard drug solution to the sample. % recovery was calculated by assaying these solutions.

**System precision, method precision and intermediate precision**

The system, method and intermediate precision of the proposed method are ascertained by injecting 6 replicates of test and standard sample, % RSD were calculated.

**Specificity**

Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded.

**Linearity**

A linear relationship was evaluated across the range of the analytical procedure. A series of standard dilutions were prepared from the working standard solution in the concentration range of 20-80 µg/ml of Rabeprazole sodium and 15-60 µg/ml of Mosapride citrate, respectively. 10 µl of each solution was injected into HPLC system. Linearity is evaluated by plotting the peak area as a function of analyte concentrations.

**Robustness**

Robustness was carried out by changing small variations in method parameters like flow rate (± 0.2 ml), wavelength (± 2 nm) and temperature (± 5 °C). Ruggedness was done by studying changes with variation of analyst.

**LOD and LOQ**

The limit of detection (LOD) and limit of quantification (LOQ) were determined for Rabeprazole sodium and Mosapride citrate.

**Procedure for forced degradation studies**

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. Further forced degradation studies were conducted for indicating the stability of the method developed. The results of the degradation studies are presented in table 2.

**Acid degradation**

481.6 mg of sample equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask and added 10 ml of 0.1N HCl. Then sonicated for 30 min & added 10 ml of 0.1N NaOH for neutralisation & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

**Base degradation**

481.6 mg of sample equivalent to 20 mg of Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of 0.1N NaOH. Then sonicated for 30 min & added 10 ml of 0.1N HCl for neutralisation & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

**Peroxide degradation**

481.6 mg of sample equivalent to 20 mg of Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of 1 % peroxide. Then sonicated for 30 min & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

**Thermal degradation**

Sample was kept in oven for 1 h at 60 °C. Above sample 481.6 mg equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of methanol. Then sonicated for 30 min & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.
UV light degradation

Sample was kept in sun light for 1 day. Above sample 481.6 mg equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of methanol. Then sonicated for 30 min & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

RESULTS AND DISCUSSION

The preliminary studies indicated that the desired system suitability parameters were obtained with the mobile phase containing methanol: buffer (ammonium acetate pH 6.5): acetonitrile in the ratio of (50:20:30 %). The mobile phase eluted the drug at lower retention times (2.951 and 4.195 min for Rabeprazole sodium and Mosapride citrate, respectively). The corresponding chromatograms were shown in the fig. 3 & 4 and the data are presented in table 1.

Table 1: System Suitability Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rabeprazole sodium</th>
<th>Mosapride citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>2.951</td>
<td>4.195</td>
</tr>
<tr>
<td>USP resolution</td>
<td>7.198194</td>
<td></td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>8102.312593</td>
<td>9879.942054</td>
</tr>
<tr>
<td>Tailing</td>
<td>1.127759</td>
<td>1.080256</td>
</tr>
</tbody>
</table>

The % RSD in precision, accuracy and robustness studies were found to be less than 2.0 %, indicating that the method is precise, accurate and robust. Accuracy data as shown in table 2.

Robustness of the method was determined by small deliberate changes in flow rate, temperature and wavelength. The low value of relative standard deviation indicates that the content of the drug was not adversely affected by these changes. Hence, the proposed method was robust. The LOD and LOQ were found to be 0.435 µg/ml and 1.319 µg/ml for Rabeprazole sodium and 0.594 µg/ml and 1.799 µg/ml for Mosapride citrate, respectively. The obtained data in validation studies are summarized in table 2. From the validation study it was cleared that all the observed values were within the acceptable range. Therefore, the method attempted to evaluate the stability of the drug under various stress conditions with different rates of decomposition. The developed method was able to detect decomposition. The chromatograms observed from samples, subjected to various stress conditions, are shown in fig. 7a to 7e. The amount of drug decomposed at various stress conditions are shown in table 2.
Table 2: Validation parameters

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Limits</th>
<th>Observation</th>
<th>Rabeprazole sodium</th>
<th>Mosapride citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specificity</td>
<td>No interference</td>
<td>No interference</td>
<td>0.2788</td>
<td>0.61058</td>
</tr>
<tr>
<td>2</td>
<td>System precision</td>
<td>RSD NMT 2.0 %</td>
<td>0.28087</td>
<td>0.79084</td>
<td>0.53061</td>
</tr>
<tr>
<td></td>
<td>Method precision</td>
<td></td>
<td>0.12861</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>Intraday precision</td>
<td>Correlation coefficient NLT- 0.999</td>
<td>0.9999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Linearity range</td>
<td></td>
<td>0.9999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Accuracy</td>
<td>% Recovery range</td>
<td>99.02-100 %</td>
<td>99.57-100.01 %</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Limit of Detection</td>
<td>Signal noise ratio should be more than 3:1</td>
<td>0.4354 µg/ml</td>
<td>0.59382 µg/ml</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Limit of Quantitation</td>
<td>Signal noise ratio should be more than 10:1</td>
<td>1.319 µg/ml</td>
<td>1.799 µg/ml</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Asymmetry factor</td>
<td>NMT 2 %</td>
<td>1.13</td>
<td>1.08</td>
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<tr>
<td>9</td>
<td>Number of Theoretical Plates</td>
<td>NLT 2500</td>
<td>7809</td>
<td>9726</td>
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<td>10</td>
<td>Robustness</td>
<td>No effect on system suitability parameters</td>
<td>No effect on system suitability parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Change in column temperature,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Change in flow rate, change in wavelength</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Degradation</td>
<td>% net degradation – 1-50 %</td>
<td>-</td>
<td>41.91 %</td>
<td>43.45 %</td>
</tr>
<tr>
<td></td>
<td>Acid</td>
<td></td>
<td></td>
<td>34 %</td>
<td>25.79 %</td>
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<tr>
<td></td>
<td>Base</td>
<td></td>
<td></td>
<td>41.11 %</td>
<td>33.68 %</td>
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<td></td>
<td>Peroxide</td>
<td></td>
<td></td>
<td>42.89 %</td>
<td>36.50 %</td>
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<td></td>
<td>Heat</td>
<td></td>
<td></td>
<td>40 %</td>
<td>25.35 %</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The proposed stability indicating RP-HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Rabeprazole sodium and Mosapride citrate in pure and its pharmaceutical dosage forms.

**CONFLICT OF INTEREST**

None to declare

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**REFERENCES**