Development and Validation of Stability Indicating HPLC Method for Estimation of Metoclopramide Hydrochloride From A Novel Formulation

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ABSTRACT

A new, simple, specific, precise and robust isocratic reversed-phase (RP) stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for determination of metoclopramide hydrochloride from a novel pellet formulation. The liquid chromatographic separation was achieved isocratically using a mobile phase of acetonitrile: water (25:75), with 0.06 % triethylamine and pH adjusted to 4 using orthophosphoric acid. The analysis was carried out using Hi-Q-Sil C18 column [250 mm x 4.6 mm, 5 µm] at flow rate of 1 ml/min and the UV detection at 274 nm. The method was validated for accuracy, precision, linearity, range, selectivity and robustness. The linearity of the proposed method was investigated in the range of 0.5–18 µg/ml ($r^2 = 0.9985$). The drug was subjected to oxidation, hydrolysis, heat and photolysis to apply stress conditions. The method provided good peak parameters with retention time of 6.2 ± 0.02 min. Degradation products resulting from stress studies did not interfere with the detection of metoclopramide hydrochloride and the assay can thus be considered as stability-indicating.

Key words: Metoclopramide Hydrochloride, reverse phase, high performance liquid chromatography, assay, validation.

INTRODUCTION

Metoclopramide hydrochloride is frequently used as an antiemetic and for treatment of gut mobility disorders. The drug has a short biological half-life and is usually administered in a dose of 10–15 mg four times daily in order to maintain effective concentrations throughout the day. However, in patients with significant degrees of renal impairment, therapy should be performed at reduced dosage (1). In long-term therapy, fluctuation in the plasma concentrations, with high concentration peaks are common for such drugs with rapid absorption and elimination (2). The secondary effects of metoclopramide on the central nervous system in the form of extrapyramidal symptoms, will surface, if plasma levels markedly exceed therapeutic levels (3). Such characteristics make metoclopramide hydrochloride a suitable candidate for controlled release delivery.

Chromatographic methods have been described for the quantitative determination of metoclopramide in formulations as well as biological fluids. These include gas chromatography (4, 5) and high performance liquid chromatography (6-10). These previous published methods comprise of complicated mobile systems and are not directly applicable for this novel type of dosage form which is prepared and need more investigation for method development and validation. Therefore, the main aim of this work was to develop and validate a stability indicating RP HPLC method for estimation of metoclopramide hydrochloride from a novel orally disintegrating tablet containing pellet formulation.

EXPERIMENTAL:

Reagents and chemicals

Metoclopramide hydrochloride was provided as gift sample from Cosme Pharmaceuticals (Mumbai, India). All the other ingredients used in formulation were obtained from S.D Fine Chemicals. Acetonitrile HPLC used was obtained from Merck (Darmstadt, Germany). Water was deionized and then doubly distilled. Other chemicals like triethylamine and orthophosphoric acid were of A.R. grade purchased from S.D. Fine chemicals Limited, India.
Instrumentation and chromatographic conditions
A JASCO HPLC system 2000 Series with Jasco PU-2080 Plus HPLC pump (Jasco Corp, Japan), equipped with a Rheodyne injection system with a 20 µl loop was used for the study. Detection was accomplished with an UV 2075 detector at 274 nm. Borwin software was used to record and evaluate the data collected during and following chromatographic analysis. The chromatographic separation was achieved at room temperature on a reverse phase column Hi-Q-Sil C18 [250 mm x 4.6 mm, 5 µm]. The mobile phase comprising of Acetonitrile: water (25:75) was delivered at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed using an ultrasonicator and the separations were achieved by isocratic elution with a flow rate of 1.0 ml/min.

Preparation of stock and standard solutions
A stock solution of metoclopramide hydrochloride (100µg/ml) was prepared by accurately weighing approximately 10 mg of metoclopramide hydrochloride into a 100 ml A-grade volumetric flask and making up to volume with double distilled water. Aliquots of the standard stock solution of metoclopramide hydrochloride were prepared with mobile phase to give the required final concentrations.

Method development and validation
A variety of mobile phases were investigated in the development of an HPLC method suitable for analysis of metoclopramide hydrochloride in the bulk drug and in the formulation. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. The method was validated according to ICH (11-14) and USP guidelines (15). The validation parameters addressed were specificity, precision, accuracy, linearity and robustness (16-23).

Stress Testing of Metoclopramide hydrochloride solution
Oxidation studies
Solutions for oxidation studies were prepared in 5 % H₂O₂ and the resultant solutions were allowed to stand for 4 h in order to facilitate oxidation of the drug.
Acid degradation studies
Solutions for acid degradation studies were prepared in 0.1 N hydrochloric acid and the resultant solutions were refluxed for 8 h.
Alkali degradation studies
Solutions for alkali degradation studies were prepared in 0.1 N sodium hydroxide and the resultant solutions were refluxed for 8 h

Neutral degradation studies
Solutions for neutral degradation studies were prepared in water and the resultant solutions were refluxed for 8 h.

Temperature stress studies
The drug was exposed to dry heat (80°C) in a hot air oven for 48 h. The drug solution was prepared and subjected to analysis.

Photostability studies
The drug was exposed to light for 48 h. The drug solution was prepared and subjected to analysis.

Preparation of sustained release orally disintegrating tablets of metoclopramide hydrochloride
The tablets were prepared by compressing sustained release pellets with sugar pellets. The pellets were formulated using pelletization method incorporating ingredients like Avicel, psyllium husk, polyvinyl pyrollidone, sodium bicarbonate and ethyl cellulose.

Assay of metoclopramide hydrochloride formulation
Tablets subjected to stability studies for a period of three months were assayed. Tablets in a number of 5 were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 15 mg of metoclopramide hydrochloride was transferred to a 100 ml volumetric flask. HCl buffer of pH 1.2 was added to make up the volume, sonicated for 15 min and filtered. From the filtrate further dilutions were made with mobile phase to get a concentration of 10 µg/ml. The resulting solutions were then injected onto the column and chromatographed using the conditions mentioned above. The percent drug content was determined from the area of the peak using the regression equation obtained in the calibration experiments. The same procedure as described above was used for the assay of marketed capsule containing pellets of metoclopramide hydrochloride.

RESULTS AND DISCUSSIONS:
Method Development
Of several solvents and solvent mixtures investigated the mobile phase consisting of acetonitrile: water (25:75), with 0.06 % triethylamine added and pH adjusted to 4 using orthophosphoric acid was found to furnish sharp, well-defined peaks with very good symmetry. The presence of triethylamine and orthophosphoric acid helped in better resolution of the peak. The retention time for the drug was 6.2 min. A typical representative chromatogram is shown in Figure 1.

Method Validation
Specificity and stress studies
The results of the stress studies indicated the specificity of the method that has been developed. After exposure of metoclopramide hydrochloride solutions to stress conditions,
an assay of the drug was performed on the resultant solutions. Typical chromatograms obtained for these analyses are shown in Figure 2 and 3. It is clearly evident that when exposed to acidic conditions and alkaline conditions the drug undergoes degradation. The peaks of the resultant degradants were well resolved from the drug peak. However the drug was found to be thermostable, photostable and stable in oxidative conditions.

The assay of a product must also show specificity with regard to the potential interference that might be a result of the presence of excipients in a formulation. The specificity studies showed that there were no extra peaks due to the excipients which indicated lack of interference from the excipients. A typical chromatogram developed during the analysis of the drug solution in presence of excipients is shown in Figure 4.

**Linearity and range**

Calibration curves that were constructed for metoclopramide hydrochloride were linear over the concentration range of 0.5 to 18 µg/ml. The peak area was plotted versus the concentration. The equation for the resultant calibration curve was \( y = 246525x + 30479 \) with a linear regression coefficient of 0.9985.

**Precision**

Intra-day precision (repeatability) and inter-day (intermediate) precision were determined. The analyses were performed using concentrations at three levels, 8, 10 and 12 µg/ml. Each concentration was analyzed in triplicate \( (n = 3) \) and intra-day precision was found to be less than 2 % R.S.D for all samples on all days. (Table I) Inter-day precision % R.S.D. for analyses was conducted on three separate days and was found to be 1.24, 1.29 and 1.67 % R.S.D. for the low, middle and high concentrations studied, respectively (Table II).

**Accuracy**

Accuracy was determined at three spiked doses of 12, 15 and 18 mg to assess the precision of the method. Each of the solutions was analyzed \( (n = 5) \) and the percentage standard deviation was determined. The method showed percentage RSD of less than 2 for all solutions tested which indicated good accuracy of the method and the results are summarized in the table III.

**Robustness**

Robustness was determined by varying the mobile phase flow rate to ± 0.2 ml/min (i.e. 0.8, 1 and 1.2 ml/min), the concentration of acetonitrile in the mobile phase to ± 2 parts (AcCN: Water as 23:77, 25:75 and 27:73) and pH of the mobile phase to ± 1 (pH 3, 4 and 5). The deliberate changes in the flow rate, mobile phase concentration and the change in pH did not affect the recovery of the drug which indicated the robustness of the method.

**Assay**

The proposed method was applied to the determination of metoclopramide hydrochloride in formulated dosage form and in a marketed preparation of the same. A typical chromatogram obtained following the assay of formulation and marketed preparations are depicted in Figure 5 and 6 respectively. The result of the assay yielded 99.24 % of label claim and is comparable to the marketed preparation. The results of the assay indicate that the method is selective for the assay of metoclopramide hydrochloride without interference from the excipients used in the dosage form.

**CONCLUSION:**

This developed RP-HPLC method for estimation of metoclopramide hydrochloride from pellets is accurate, precise, robust, specific, and stability-indicating. The method has been found to be better than previously reported methods, because of its less retention time, use of an economical and readily available mobile phase, UV detection and better resolution of peaks. The run time is relatively short, which will enable rapid quantification of many samples in routine and quality-control analysis of various formulations containing metoclopramide hydrochloride. All these factors make this method suitable for quantification of metoclopramide hydrochloride in bulk drugs and in pharmaceutical dosage forms without any interference. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating.

**ACKNOWLEDGEMENTS:**

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Figure 1: Typical standard chromatogram of metoclopramide hydrochloride

Figure 2: Chromatogram obtained following the exposure of drug to acidic conditions (0.1N HCl)

Figure 3: Chromatogram obtained following the exposure of drug to alkaline conditions (0.1N NaOH)

Figure 4: Typical Chromatogram obtained following analysis of metoclopramide hydrochloride in presence of excipients
Table I: Intra-day precision

<table>
<thead>
<tr>
<th>Levels (µg/ml)</th>
<th>Concentration Recovered (µg/ml)</th>
<th>% RSD</th>
<th>Concentration Recovered (µg/ml)</th>
<th>% RSD</th>
<th>Concentration Recovered (µg/ml)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>8.05 ± 0.09</td>
<td>1.11</td>
<td>8.03 ± 0.10</td>
<td>1.24</td>
<td>8.07 ± 0.05</td>
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<td>10</td>
<td>7.95 ± 0.02</td>
<td>0.29</td>
<td>9.49 ± 0.07</td>
<td>0.73</td>
<td>9.50 ± 0.08</td>
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<tr>
<td>12</td>
<td>8.12 ± 0.06</td>
<td>0.53</td>
<td>11.50 ± 0.10</td>
<td>0.86</td>
<td>11.56 ± 0.06</td>
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</table>

Table II: Inter-day precision

<table>
<thead>
<tr>
<th>Levels (µg/ml)</th>
<th>Concentration Recovered (µg/ml)</th>
<th>% RSD</th>
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</thead>
<tbody>
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<td>8</td>
<td>7.94 ± 0.09</td>
<td>1.24</td>
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<tr>
<td>10</td>
<td>9.57 ± 0.12</td>
<td>1.29</td>
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<tr>
<td>12</td>
<td>11.86 ± 0.19</td>
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SD – Standard Deviation, RSD – Relative standard deviation

<table>
<thead>
<tr>
<th>Amount added (mg)</th>
<th>Amount Recovered (mg)</th>
<th>Percentage Recovered</th>
<th>S.D</th>
<th>% RSD</th>
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<td>12</td>
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<td>101.5</td>
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<td>0.52</td>
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</tbody>
</table>

SD – Standard Deviation, RSD – Relative standard deviation

Figure 5: Chromatogram of metoclopramide in tablet assay at the end of three months

Figure 6: Chromatogram of metoclopramide in marketed tablet


13. Q 2 (R1) ICH guidelines, Validation of Analytical Procedures: Text and Methodology.

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