A Validated RP-HPLC Method for Estimation of Metoclopramide Hydrochloride and Paracetamol in solid dosage form

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ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of Metoclopramide hydrochloride and Paracetamol in combined dosage form by RP-HPLC method. RP-HPLC estimation of drugs in selected combination was done using Phenomenex ODS 5µ, C₈ column (250x4.6mm) and ACN: Methanol: (0.5%) TEA Buffer (18.5:6.5:75) as mobile phase which shows sharp and resolved peak when detected at 273nm. The linearity range was found to be in concentration range 10-50 µg/mL for Metoclopramide (MET) and 10-50 µg/mL for Paracetamol (PAR). The retention time for Paracetamol and Metoclopramide were 4.1 and 5.9 respectively. The correlation coefficient was found to be 0.9999 (for MET) and 0.9992 (for PAR). The mean percentage recovery was found to be 98.69 and 98.92 for MET and PAR respectively. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. Validation of the proposed method was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed method can be successfully applied in routine work for the determination of Metoclopramide and Paracetamol in combined dosage form.

Key words: Metoclopramide hydrochloride, Paracetamol, RP-HPLC method.

INTRODUCTION


EXPERIMENTAL

Instrument
Shimadzu HPLC 1100 series chromatograph equipped with binary pump LC-10 ADvp, SPD-10 UV detector, Rheodyne Manual injector 7725i with 20µL loop and a reversed phase 5µ Phenomenex ODS C18 column (250 x 4.6 mm) with pore size of 100Å was used for the chromatographic studies. Shimadzu AUX220 balance was used for weighing the samples. All the chemicals used were of HPLC grade. Double distilled water and Whatmann filter paper (no.41) were used throughout the experimental work.

Materials
Multicomponent tablet METOPAR (MET 5mg and PAR 500mg) manufactured by cosme farma laboratories limited, Karnataka. All chemicals used were of analytical grade, and HPLC grade acetonitrile (Merck, Ltd, Mumbai) were used. Double distilled water filtered through 0.45µm filter (MILLI PORE) was used to prepare solutions.

Chromatographic conditions
Chromatographic Separation was achieved on ODS Phenomenex C-18 (250mm x 4.6 mm, 5µ) column. The mobile phase consisting of Acetonitrile, Methanol and (0.5%) TEA Buffer (18.5: 6.5:75) adjusted to pH3.0 with orthophosphoric acid, was delivered at rate of 1.0mL/minute. The mobile phase was filtered through 0.45µm membrane filter (Millipore) and degassed prior to use. Separation was performed at ambient temperature i. e. 30°C and detection was made at 273 nm. The injection volume was 20 µL with a run time of 10 min.

Preparation of standard solution
i) Paracetamol (PAR) standard stock solution: [S₁]
An accurately weighed quantity of about 25.0 mg Paracetamol in 50ml volumetric flask, dissolved in sufficient quantity of mobile phase (as mentioned below) and volume was made up to the mark with mobile phase. (Concentration: 500µg/mL of PAR).

ii) Metoclopramide hydrochloride (MET) standard stock solution: [S₃]
An accurately weighed quantity of about 25.0 mg Paracetamol in 50ml volumetric flask, dissolved in sufficient quantity of mobile phase (as mentioned below) and volume was made up to the mark with mobile phase. (Concentration: 500µg/mL of MET).

iii) Mixed standard solution:
Accurately weighed quantities of about 25.0 mg PAR and 25.0 mg MET were transferred to a 50.0 mL volumetric flask, dissolved in sufficient quantity of mobile phase and volume was made up to the mark with mobile phase. (Concentration: 500µg/mL for PAR, 500µg/mL for MET). [S₄] A 1.0 mL portion of the above mixed standards solution was diluted up to 50.0 mL with mobile phase containing Acetonitrile, methanol and (0.5%) TEA buffer in the ratio 18.5:6.5:75 v/v to get the concentration of 10µg/mL for MET and 10µg/mL for PAR respectively (Concentration:10 µg/mL PAR, 10 µg/mL MET). [S₅]

Methods:
The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Column: Phenomenex ODS 5 µ C18 column (250 X 4.6mm)
Detection Wavelength : 273 nm
Flow rate : 1.0 mL/min
Temperature: Ambient : (28–30°C)

pH : 3.0

A 20 µL solution of above mix standard was injected through manual injector and chromatogram was recorded using mobile phase containing Acetonitrile, Methanol and (0.5%) TEA Buffer (18.5: 6.5:75). Metoclopramide and Paracetamol were resolved properly with sharp peak and showing reasonable retention time in the above selected mobile phase. A chromatogram for both drugs so recorded in shown in fig 1.

Study of system suitability parameters
After equilibration of column with mobile phase, seven replicate injections of 20 µL solution of mix standard solution was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

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Table 1: System Suitability Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suitable Values</th>
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<tbody>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile, methanol and TEA Buffer (18.5:6.5:75)</td>
</tr>
<tr>
<td>Retention time</td>
<td>4.217 5.923</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.417 1.548</td>
</tr>
<tr>
<td>Resolution</td>
<td>8.405</td>
</tr>
<tr>
<td>Theroretical plate/column</td>
<td>9115 10407</td>
</tr>
<tr>
<td>Theroretical plate/meter</td>
<td>9115104068</td>
</tr>
</tbody>
</table>

Table 2: Results of Marketed formulations and Recovery study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean % label claim* ± S.D.</th>
<th>Mean % Recovery** ± CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR</td>
<td>101.31 ±0.75</td>
<td>98.69 ± 0.53</td>
</tr>
<tr>
<td>MET</td>
<td>100.28 ±0.44</td>
<td>98.92 ± 0.81</td>
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</table>

Table 3: Forced degradation study

<table>
<thead>
<tr>
<th>% Label claim</th>
<th>Condition</th>
<th>1N NaOH</th>
<th>1N HCl</th>
<th>6% H₂O₂</th>
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<tbody>
<tr>
<td>PAR</td>
<td>Water</td>
<td>91.70</td>
<td>63.62</td>
<td>158.45</td>
</tr>
<tr>
<td>MET</td>
<td>98.32</td>
<td>83.73</td>
<td>66.07</td>
<td>87.10</td>
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</table>

Table 4: Intermediate precision and Ruggedness study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean % label claim ± S.D.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PAR</td>
</tr>
<tr>
<td>Different Analyst (n=3)</td>
<td>100.79 ±0.61</td>
</tr>
<tr>
<td>Intraday Variation (n=3)</td>
<td>100.79 ±0.61</td>
</tr>
<tr>
<td>Inter day Variation (n=3)</td>
<td>100.79 ±0.62</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>12µg/ml</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>3 µg/ml</td>
</tr>
<tr>
<td></td>
<td>MET</td>
</tr>
<tr>
<td>Different Analyst (n=3)</td>
<td>100.04 ±0.63</td>
</tr>
<tr>
<td>Intraday Variation (n=3)</td>
<td>100.04 ±0.61</td>
</tr>
<tr>
<td>Inter day Variation (n=3)</td>
<td>99.53 ±0.72</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>8µg/ml</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>2 µg/ml</td>
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</table>

Study of Linearity Range

Aliquots of mixed standard stock solution were diluted in range 1.0 mL to 5.0 mL in 50 mL volumetric flask with mobile phase, volume was made up to mark with mobile phase to obtain concentration 10µg/mL to 50µg/mL for MET and 10µg/mL to 50µg/mL for PAR respectively. The graphs of concentration of drug vs. area under curve were plotted for both the drugs. The correlation coefficient was found to be 0.9989 for MET and 0.9992 for PAR.

Limit of detection and limit of quantitation

The LOD was calculated to be 3µg/mL for Paracetamol and 3µg/mL for Metoclopramide hydrochloride.
Metoclopramide. And the LOQ of Paracetamol and Metoclopramide were found to be 10 µg/mL and 10 µg/mL, respectively. Chromatographic parameters, such as number of theoretical plates (N), resolution (Rs), capacity factor (k) and selectivity factor (a) were determined. The results are shown in (Table 4), indicating the good performance of the system.

**Assay in Marketed Formulation**

An accurately weighed quantity of tablet powder equivalent to 25.0 mg of MET (~25.0 mg of PAR) was transferred to 50.0 mL volumetric flask, sonicated for 30 minutes with sufficient quantity of mobile phase and volume was made up to mark with mobile phase. The contents of the flask were filtered through Whatmann filter paper (no.41). A 1.0 mL portion of the filtrate was further diluted to 50.0 mL with a mobile phase. The sample solution was injected and the chromatogram was recorded. The content of MET and PAR were calculated by comparison of the standard area and sample area and results are shown in Table 2. MET present in this tablet powder was 0.25 mg, which could not be found accurately due to low area; hence to increase the accuracy, accurately weight 24.75 mg pure drug sample of MET was transferred to the the same volumetric flask.

**VALIDATION**

**Accuracy:**

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by proposed method and the mean % recovery were found to be 98.69 and 98.92 for PAR and MET respectively.

**Precision:**

System precision is the measure of the method variability that can be expected for a given analyst performing the analysis. Precision of the method was determined with the product. An amount of the product powder equivalent to 25, 50 and 75% of label claim of PAR and MET were calculated with the standard area and sample area and results are shown in Table 3, indicating that acceptable precision was achieved for Metoclopramide hydrochloride and Paracetamol, as revealed by relative standard deviation data (RSD<2.0% in all of the levels of the two drugs).

**Linearity and Range:**

Accurately weighed quantities of tablet content equivalent to about 80, 90, 100, 110 and 120% of label claim of PAR were taken and dilutions were made as described under marketed formulation. The chromatograms of the resulting solutions were recorded. The plot of AUC Vs Percent label claim was found to be linear with correlation coefficient of 0.9996 for PAR and 0.9984 for MET.

**Stability studies:**

The forced degradation studies were carried out at 50°C using 1 ml of 1N NaOH, 1N HCL, 6% H2O2 and the chromatograms recorded are shown in fig 2,3,4 respectively. Volumes were made up to the mark with mobile phase, further aliquots were diluted with mobile phase and sample solutions were injected separately and chromatograms under stress conditions were recorded. The results showed slight difference in the percent label claim as compared with normal condition. In all the stress condition Paracetamol was found to be more sensitive to hydrolysis and oxidation. The results are shown in Table 3. Precision and Intermediate precision (Intra day and Inter day) shows the % Label claim values within limits (% R.S.D. not more than 2). The method was found to be precise. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision and ruggedness parameter are shown in Table 4.

**RESULTS AND DISCUSSION**

The optimised chromatographic conditions gave well resolved and sharp peaks of PAR and MET with retention times 4.197 and 5.910 respectively. It was observed that the proposed method can be easily applied to marketed formulation and the statistical parameter viz. S.D., CV is in the acceptable range for quantitative determination of PAR and MET. The method validation parameter like accuracy, precision, Linearity and range and specificity were found to be satisfactory.

**CONCLUSION**

The results obtained by the proposed method for determination of PAR and MET are reliable, accurate and precise. The values of standard deviation were found satisfactory and the recovery studies were close to 100%. The method does not require prior separation of one drug from other. Hence it can be employed for routine quality control analysis of PAR and MET in combined dosage form.

**REFERENCES**