Spectrophotometric Estimation of Moclobemide Using Folin Ciocalteu’s Reagent

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Abstract: A specific, accurate and precise spectrophotometric method using Folin Ciocalteu’s reagent for determination of moclobemide in tablet dosage form has been established and validated. Intense color was produced in the presence of 1.0 ml of 20% Na₂CO₃ solution and 3.0 ml of FC reagent solution (1:1 diluted with water) and wavelength maxima was 778 nm. Linearity over 10-200 µg/ml with r² = 0.9982 was observed. %RSD for precision on replication was 0.1108 for seven replicate analyses. %RSD for intra-day and inter-day precision of moclobemide for 10-200 µg/ml was 0.128-0.495 and 0.155-0.670, respectively. Detection limit (LOD) and quantification limit (LOQ) determined mathematically were 1.311µg/ml and 3.974µg/ml, respectively. % recovery was 98.578 – 100.269 ± 0.245-0.612. Statistical analysis proves the method is repeatable and specific for analysis of moclobemide.

INTRODUCTION
Moclobemide, 4-chloro-N-(2-morpholinoethyl) benzamide is a potent, specific monoamine oxidase-A (MAO-A) inhibitor. It inhibits the deamination of serotonin, norepinephrine and dopamine. This action leads to increased concentrations of these neurotransmitters, which may account for the depressant activity of moclobemide. There is not any official method for moclobemide. Various analytical methods, for example use of an ion-selective electrode(1), spectrophotometric analysis by charge-transfer complexation(2), LC(3) and derivative spectrophotometric(4) have been used for estimation of moclobemide as active pharmaceutical ingredient and in dosage forms. HPTLC has been used for analysis of moclobemide in the presence of other antidepressants(5,6).

EXPERIMENTAL
Materials
Pharmaceutical grade moclobemide was kindly gifted by Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. All chemicals and reagents were of analytical grade. The
dosage form of moclobemide was procured from the local pharmacy.

**Instrumentations**
A shimadzu model 1601 double beam UV-visible spectrophotometer with a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solutions. A Sartorius CP224S analytical balance was used to weigh the materials.

**Methodology**
FC reagent was diluted with distilled water. Sodium carbonate solution was prepared with distilled water. A stock solution of moclobemide was prepared by dissolving 10 mg of moclobemide in distilled water and diluted up to the 10 ml with distilled water. Experimentation was performed at room temperature. Factors affecting the intensity of colored chromogen like concentration (10%, 15%, 20% and 25%) and volume (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml) of sodium carbonate; and concentration (25%, 50%, 75% and 100%) and volume (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml) of FC reagent were studied keeping one variable constant.

**Optimization of the method**
To estimate the moclobemide content of a tablet (label claim 150 mg), 20 tablets were accurately weighed and powdered and the powder equivalent to 100 mg of moclobemide was transferred to a 100 ml volumetric flask and mixed with distilled water (50 ml) and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with distilled water. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with distilled water. 1.0 ml of this solution was transferred to 10.0 ml of volumetric flask. 1.0 ml of sodium carbonate solution and 3.0 ml of FC reagent was added and mixed. The mixture was kept aside for 10 minutes for the development of color and the volume in each flask was adjusted to 10 ml with distilled water. The absorbance of solution was measured at 778 nm against reagent blank. The analysis was repeated for three times.

**RESULTS AND DISCUSSION**

**Optimization of the method**
In the proposed method, standard stock solution of moclobemide was prepared in distilled water. Various reaction conditions were established by varying one parameter at a time and keeping the other fixed by observing the effect produced on the absorbance at the colored species. Maximum absorbance was observed in the presence of 1.0 ml of 20% Na₂CO₃ solution and 3.0 ml of FC reagent solution at 778 nm (fig. 1, 2, 3 & 4).

**Limit of Detection and Limit of Quantification**
The limit of detection (LOD) and limit of quantification (LOQ) were estimated mathematically. The mathematical formulas used were:

LOD = 3.3 x (standard deviation/slope of the calibration plot)
LOQ = 10 x (standard deviation/slope of the calibration plot)
Spectrophotometric Estimation of Moclobemide

(LOQ) were 1.311µg/ml and 3.974µg/ml, respectively. % recovery (98.578 – 100.269 ± 0.245-0.612) reveals that excipients usually present in the pharmaceutical formulation do not interfere. Method validation data are summarized in table 1. The results of the analysis of pharmaceutical dosage form by the proposed method are highly reproducible and in good agreement with labeled claim of the drug (table 2).

CONCLUSION
As moclobemide contains nitrogen in the structure, it acts as reducing agent and reduces tungstate and molybdate which are present in FC reagent in alkaline medium provided by sodium carbonate and forms blue color. Color formation provides more specificity to this method. The proposed method is simple, sensitive, accurate and precise over wide range 10-200 µg/ml; and can be used for the routine analysis of moclobemide in pharmaceutical dosage form.

Table 1 Summary of validation parameters:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>10-200 µg/ml</td>
</tr>
<tr>
<td>Correlation co-efficient</td>
<td>0.9982</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>0.1107</td>
</tr>
<tr>
<td>Repeatability (n=7)</td>
<td>0.128-0.498</td>
</tr>
<tr>
<td>Intra-day precision (n=3)</td>
<td>0.155-0.670</td>
</tr>
<tr>
<td>Inter-day precision (n=3)</td>
<td>0.128-0.498</td>
</tr>
<tr>
<td>Accuracy (%recovery ± RSD)</td>
<td>98.578-100.269 ± 0.245-0.612</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>1.311 µg/ml</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>3.974 µg/ml</td>
</tr>
</tbody>
</table>

Figure 1 Optimization of ml of sodium carbonate (20%) solution:

Figure 2 Optimization of ml of FC reagent:

Figure 3 Optimization of concentration of sodium carbonate solution
Table 2 Estimation of moclobemide in tablet:

<table>
<thead>
<tr>
<th>Formulation (tablet)</th>
<th>Labeled amount</th>
<th>Average amount found</th>
<th>% assay ± S.D. (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand-1</td>
<td>150 mg</td>
<td>149.015 mg</td>
<td>99.343 ± 0.169</td>
</tr>
<tr>
<td>Brand-2</td>
<td>150 mg</td>
<td>149.897 mg</td>
<td>99.931 ± 0.169</td>
</tr>
</tbody>
</table>

Figure 4 optimization of concentration of FC reagent

REFERENCES