PHARMAGENE
Vol:1 Issue:1
Research Article
www.genesisjournals.org

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% Na_2CO_3 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 r^2 = 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.

Received on 07-01-2013 **Modified on 18-01-2013** **Accepted on 07-02-2013**

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)].

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GC-MS and LC-PDA have been used after solid-phase extraction from blood [(7)] and LC-ESI-MS [(8),] LC [(9)] and LC-UV methods [(10-11)] have been used, after extraction, for analysis of moclobemide in human plasma. Stability indicating HPTLC method has been reported [(12)].

Any colorimetric method is not reported for estimation of moclobemide. The objective of the work discussed in this paper was to establish an accurate, specific and precise colorimetric method for estimation of moclobemide as an active pharmaceutical ingredient and in tablet. In this method, drug act as reducing agent and reduce Folin Ciocalteu's reagent in alkaline medium and produce blue color. Folin Ciocalteu's (FC) reagent consists of sodium tungstate, sodium molybdate, phosphoric acid, hydrochloric acid, lithium sulphate, bromine and water [(13)]. The proposed method was as per ICH guideline [(14)].

EXPERIMENTAL

Materials

Pharmaceutical grade moclobemide was kindly gifted by Intas Pharamaceuticals 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.

Determination of wavelength for maximum absorbance

1.0 ml standard stock solution of moclobemide, 1.0 ml 20% sodium carbonate solution and 3.0 ml FC reagent (1:1 diluted with water) was mixed in 10 ml volumetric flask. The mixture was kept a side for 10 minutes and the volume was adjusted to 10 ml with distilled water. The absorbance of the colored solution was scanned against reagent blank in visible region. Maximum absorbance was obtained at 778 nm

Calibration

Accurately measured standard stock solution (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6 and 2.0 ml) were transferred to a series of 10 ml volumetric flasks. To each flask, 1.0 ml of 20 % sodium carbonate solution and 3.0 ml of FC reagent were added and mixed. The mixture was kept aside for 10 minutes for the development of colour and the volume in each flask was adjusted to 10 ml with distilled water. The absorbance was measured at 778 nm against reagent blank.

Method Validation

Precision

System intra-day repeatability was determined by measuring absorbance of the solution (100 µg/ml) against reagent blank. The absorbance of the solution was measured seven times and % RSD was calculated. Intraday and interday precision were determined by analyzing moclobemide (10-200 µg/ml) for 3 times in the same day and daily for 3 days, respectively.

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)

Specificity

The specificity of the method was ascertained by analyzing drug standard and sample. The presence of moclobemide in sample was confirmed by comparing the λ_{max} and spectra of the sample with that of the standard.

Accuracy

Accuracy was determined in terms of percentage recovery. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets with four different concentration of standard.

Analysis of moclobemide in marketed formulation

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 filterate 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% Na_2CO_3 solution and 3.0 ml of FC reagent solution at 778 nm (fig.1,2, 3 & 4).

Validation of the method

Absorbance and concentration were subjected to least squares linear regression analysis to calculate the calibration equation and correlation coefficient. Calibration plot was linear over 10-200 µg/ml (r^2 = 0.9982, slope = 0.0034, intercept= 0.1459). %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) 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:

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

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

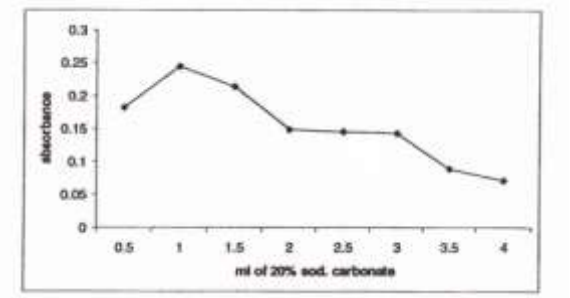


Figure2 Optimization of ml of FC reagent:

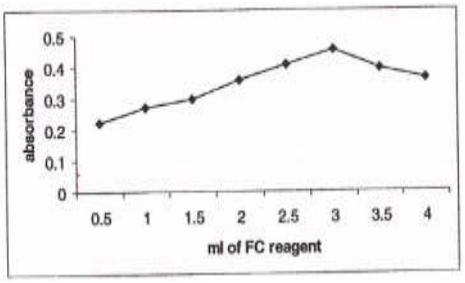


Figure 3 optimization of concentration of sodium carbonate solution

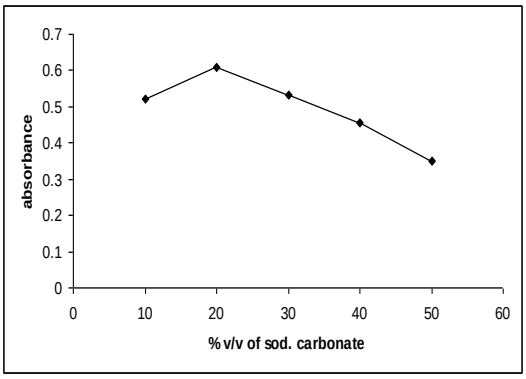
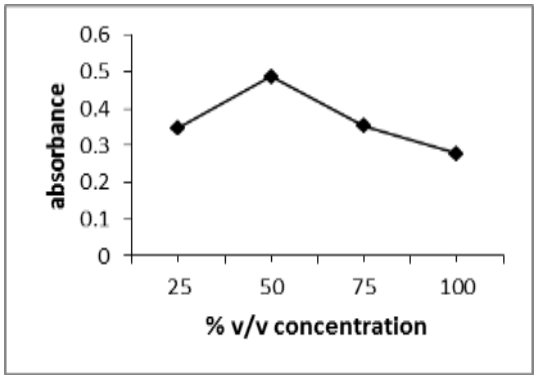


Table 2 Estimation of moclobemide in tablet:

Formulation (tablet)	Labeled amount	Average amount found	% assay ± S.D. (n=3)
Brand-1	150 mg	149.015 mg	99.343 ± 0.169
Brand-2	150 mg	149.897 mg	99.931 ± 0.169

Figure 4 optimization of concentration of FC reagent



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