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Development of New Analytical Method and its Validation for the Determination of Metoclopramide Hydrochloride in Bulk and Marketed Formulation

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ABSTRACT

In the present work, simple, sensitive, rapid and accurate analytical methods have been developed for the estimation of Metoclopramide hydrochloride in bulk and pharmaceutical dosage form. Colorimetric method for Metoclopramide hydrochloride was based on reaction involving the formation of greenish blue color complex between Metoclopramide hydrochloride and 0.02% malachite green in the presence of 0.01M chloramine-T and 2M H_2SO_4, which obeyed Beer's law in the concentration range of 2-10 µg/ml at λmax of 623nm. The molar absorptivity and sandell's sensitivity was found to be 1.669×10^2 and 0.01739. The method was validated according to ICH guidelines. The regression equation was 0.056x + 0.003. The correlation coefficient was found to be 0.999.

Keywords: Metoclopramide hydrochloride, Chloramine-T, Malachite green, H_2SO_4

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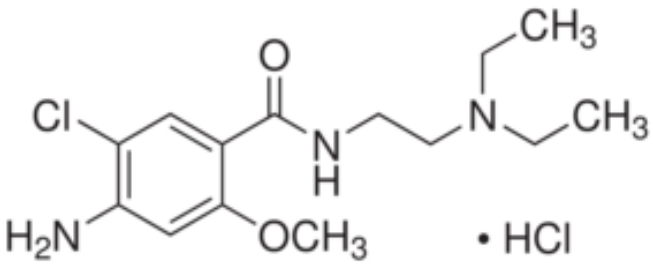
INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample [1].

Metoclopramide hydrochloride is an antiemetic and gastroprokinetic agent. It is a medicine which is used in surgical procedures as premedication, nausea and vomiting, procedures for investigating gastrointestinal disorders and relieving symptoms of gastroduodenal dysfunction. It works by increasing the action of the muscles in the gastrointestinal tract, so that food moves more quickly from the stomach to the lower gut.

Clinicians commonly use Metoclopramide hydrochloride to treat nausea - including that due to chemotherapy and that occurring post-operatively. Evidence also supports its use for gastroparesis (poor stomach emptying) and gastroesophageal reflux disease [2].



IUPAC name of metoclopramide hydrochloride is 4-amino- 5-chloro-N-(2-(diethylamino)ethyl)-2- methoxybenzamide hydrochloride. Molecular formula is $C_{14}H_{22}ClN_3O_2 \cdot HCl$ and molecular weight is 336.26 [3].

It is freely soluble in water and alcohol, sparingly soluble in methylene chloride [3].

MATERIALS AND METHODS

Metoclopramide hydrochloride was determined spectrophotometrically in bulk and marketed formulation by using malachite green as dye and chloramine-T as a strong oxidizing agent in presence of H_2SO_4.

EXPERIMENTAL

Instrumentation

All experiments were performed in JASCO V-630 series UV spectrophotometer and Shimadzu 1700 with 1 cm path length matched glass cuvettes.

Preparation of standard stock solution of Metoclopramide hydrochloride

Standard stock solution was prepared by accurately weighing 100 mg of Metoclopramide hydrochloride in 100 ml calibrated volumetric flask and made up the volume with ethanol up to 100 ml to get concentration of 1000µg/ml.

Preparation of working standard solution of Metoclopramide hydrochloride

Working standard was prepared by transferring 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with ethanol to get concentration of 100µg/ml.

Preparation of Reagents

Preparation of 0.01M Chloramine-T solution

Weighed accurately 280 mg of Chloramine-T and transferred into 100 ml volumetric flask and made up the volume with distilled water.

Preparation of 2M H_2SO_4

Transferred 10.8 ml of concentrated H_2SO_4 into 100 ml volumetric flask and made up the volume with distilled water.

Preparation of Malachite green (0.02%)

Weighed accurately 20 mg of Malachite green and added in 100 ml volumetric flask then diluted upto 100 ml with distilled water.

Determination of Absorption Maximum

An absorption maximum (or) max are the Wavelength at which maximum absorption takes place. It is important to know the absorption maximum of the substance under study, since it helps to avoid any interfering impurities.

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Procedure

0.5 ml of 0.01M chloramine-T solution, 1 ml of 2M H_2SO_4, 0.8 ml of the Metoclopramide hydrochloride working standard stock solution were added to the 10 ml volumetric flask. It was kept aside for 10 minutes for completion of reaction. Added 0.2 ml of 0.02% malachite green and kept aside for 5 minutes for completion of reaction and made up the volume with ethanol. Absorbance against reagent blank was recorded.

These solutions were scanned in UV spectrophotometer between 400-800 nm. The graph was recorded in figure no.1

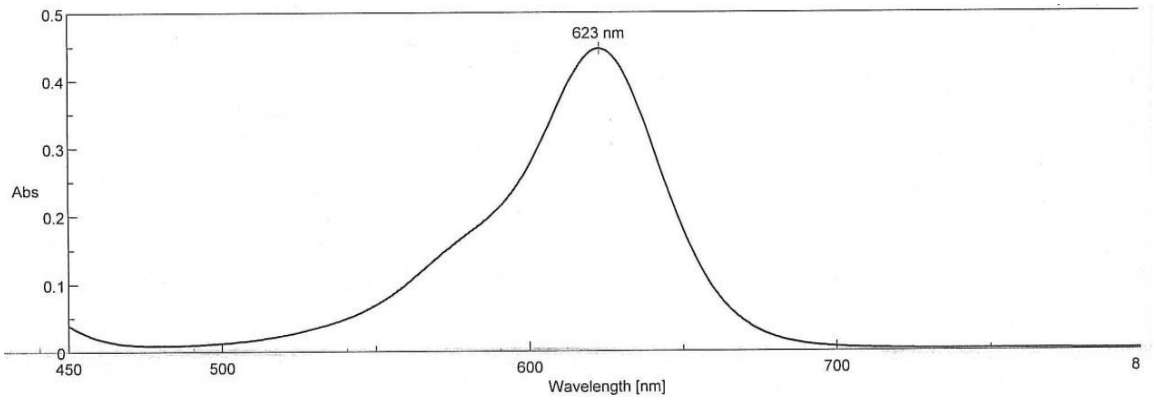

Figure no. 1 λmax graph for Metoclopramide hydrochloride with chloramine-T and malachite green

Model: JASCO V-630
Band width: 1.5 nm
Measurement: 800-400 nm
λmax: 623 nm
Absorbance: 0.450

Study of Beer-Lambert's Law

Standard curve was prepared by using pure Metoclopramide hydrochloride the concentration range of 2-10 µg/ml by this method and selecting absorption maximum at 623 nm.

Procedure

0.5 ml of 0.01M Chloramine-T, 1 ml of 2M H_2SO_4 were taken in 5 volumetric flasks of 10 ml. 0.2, 0.4, 0.6, 0.8, 1 ml of working standard of Metoclopramide hydrochloride were added in each volumetric flask and kept aside for 10 minutes. 0.2 ml 0.02% of malachite green was added and kept aside for 5 minutes for completion of reaction and made up the volume with ethanol. Absorbance was taken against reagent blank at 623 nm. The result was recorded in table no.1 and figure no.2

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Table 1: Absorbance of different concentrations of Metoclopramide hydrochloride at 623nm

Sr. No.	Vol. of working standard drug	Conc. Of drug (µg/ml)	Absorbance
1	0.2 ml	2	0.115
2	0.4 ml	4	0.230
3	0.6 ml	6	0.347
4	0.8 ml	8	0.454
5	1.0ml	10	0.561

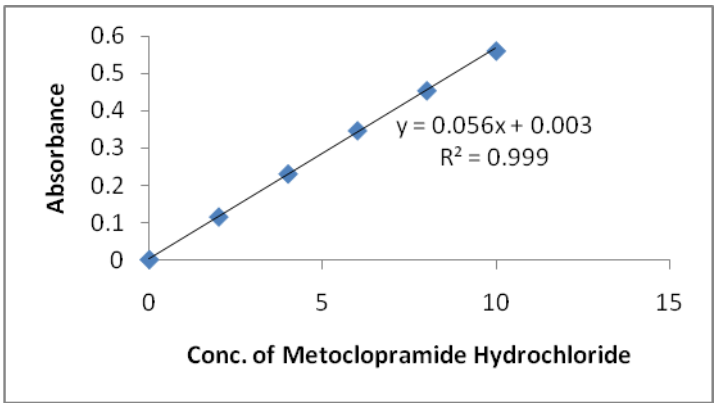

Figure no.2. Standard Curve and linearity curve for Metoclopramide hydrochloride

Analysis of Marketed Formulation

Metoclopramide HCl is marketed as Perinorm 10 mg tablet manufactured by Ipca Laboratories was taken for analysis.

Reagent and chemicals

- Working standard stock solution (100µg/ml)
- 0.01M Chloramine-T solution
- 2M H_2SO_4
- 0.02% Malachite green

Preparation of sample solution

10 tablets were weighed and crushed properly using a mortar and pestle. Then powder weight equivalent to 100mg was weighed and transferred to 100ml of volumetric flask and dissolved in ethanol and filtered through whatmann filter paper in to another 100ml volumetric flask and made up to mark with same diluent which give the solution of 1000µg/ml conc. Again 10 ml of solution was taken in 100 ml volumetric flask to get conc.,of 100µg/ml. Further dilution was performed to get a concentration of 10µg/ml. The result was recorded in table no.2

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Table 2: Assay result of marketed formulation of Metoclopramide hydrochloride

Formulation	Actual Concentration Of Metoclopramide Hydrochloride (µg/ml)	Amount Obtained Of Metoclopramide Hydrochloride (µg/ml)	% of Metoclopramide Hydrochloride
Tablet	10 µg/ml	9.957 µg/ml	99.57 %

Method Validation

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance (by dilution of a standard stock solution) and using the proposed procedure. This method obeys the Beer- Lambert's law in the concentration range of 2-10 µg/ml.

Accuracy of recovery studies

The accuracy of the methods was determined by calculating % recovery of Metoclopramide hydrochloride by standard addition method. Known volumes of standard solutions of Metoclopramide hydrochloride were taken for recovery studies in 3 different levels 50%, 100%, 150% and recovery study was carried out. The result was recorded in table no.3

Table 3: Accuracy study of Metoclopramide hydrochloride

Drug	Amount Present In Formulation (µg/ml)	Amount Added (%)	Amount Recovered µg/ml	% Recovery
Metoclopramide Hydrochloride	10	-	9.957	-
		50	4.91	98.33%
		100	9.96	99.60%
		150	14.88	99.24%

Method precision (% Repeatability)

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 6 µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable**.** The result was recorded in table no.4

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3 σ/S
LOQ = 10 σ/S

Where, σ = the standard deviation of the response,
S = slope of the calibration curve.

Table 4: Method Precision (% Repeatability) of Metoclopramide hydrochloride

Conc. in µg/ml	Absorbance
6	0.342
6	0.343
6	0.342
6	0.339
6	0.340
6	0.341
Mean	0.341
SD	0.001483
%RSD	0.4349 ± 0.001211

Table 5: LOD and LOQ for Metoclopramide hydrochloride

Drug	LOD (µg/ml)	LOQ (µg/ml)
Metoclopramide Hydrochloride	0.0873	0.2648

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed methods were performed by analysing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Metoclopramide Hydrochloride (6, 8, 10 µg/ml). The results were reported in terms of relative standard deviation (RSD). The result was recorded in **table no.6** and **table no.7**

Table 6: Intermediate Precision (Interday) Of Metoclopramide hydrochloride

Conc. (µg/ml)	Day	Absorbance	Mean (X)	%RSD = 100 SD/X	Acceptance criteria
6	1	0.346	0.346	0.5780 ± 0.00230	% RSD < 2
	4	0.344			
	7	0.348			
8	1	0.457	0.457	0.3459 ± 0.00182	
	4	0.459			
	7	0.456			
10	1	0.564	0.563	0.4699 ± 0.00305	
	4	0.565			
	7	0.560			

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Table 7: Intermediate Precision (Intraday) Of Metoclopramide hydrochloride

Conc. (µg/ml)	Time (hours)	Absorbance	Mean (X)	%RSD = 100 SD/X	Acceptance criteria
6	2	0.346	0.344	0.6167 ± 0.00244	% RSD < 2
	4	0.343			
	6	0.342			
8	2	0.457	0.455	0.5815 ± 0.00305	
	4	0.455			
	6	0.452			
10	2	0.564	0.561	0.5494 ± 0.00355	
	4	0.562			
	6	0.558			

Table 8: Statistical data for Metoclopramide hydrochloride by colorimetric method

Parameter	Result
λmax (nm)	623 nm
Beer's law limits (µg/ml)	2-10 µg/ml
Sandell's sensitivity (µg.cm^2/ 0.001 Au)	0.01739
Molar absorptivity (1/mol.cm)	1.669×10^2
Regression equation (y=a+bc) Slope (b) Intercept (a)	 0.056 0.003
Correlation coefficient(R^2)	0.999
% Recovery	99.57%
%RSD	0.4349
LOD	0.0873
LOQ	0.2648

RESULT AND DISCUSSION

The objective of the proposed work was to develop new analytical methods for the determination of Metoclopramide hydrochloride and validate the methods according to ICH guidelines and applying the same for its estimation in marketed formulations.

Developed colorimetric method was found to be rapid, simple, precise, accurate and economic for routine estimation of Metoclopramide Hydrochloride in commercial dosage forms.

Estimation of Metoclopramide hydrochloride is based on oxidation reaction. In this method chloramine- T is used as oxidizing agent in presence of H_2SO_4. After completion of reaction known quantity of malachite green is added. Part of malachite green is oxidized by reacting with excees of chloramine-T and remaining part gives greenish blue color. Color of the solution indicates the amount of drug present. The solution was analysed at λmax 623 nm.

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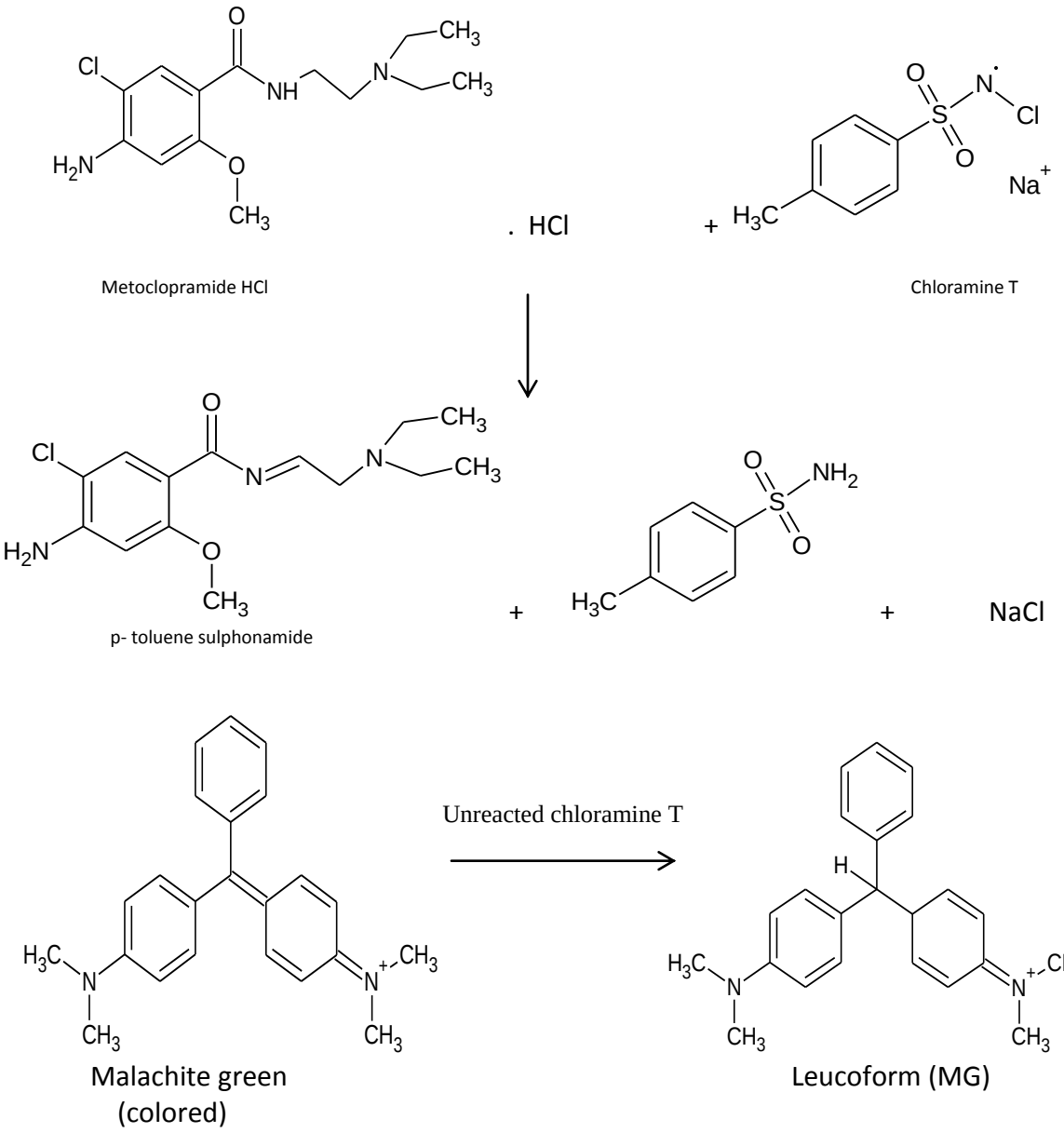

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing large number of samples in a short time period with due accuracy and precision.

Metoclopramide hydrochloride is official in Indian Pharmacopoeia. A very few analytical methods appeared in the literature for the determination of Metoclopramide hydrochloride HPLC, HPTLC, and UV-Visible spectrophotometric methods. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation, colorimetric method for the quantitative estimation of Metoclopramide hydrochloride in bulk drug and pharmaceutical formulations has been developed.

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