IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2023 Vol.:28, Issue:1

Validated Spectrophotometric Method for the Estimation of Metoclopramide in Pure and Pharmaceutical Formulation

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ISSN 2349-7203
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Submitted: 25 July 2023
Accepted: 18 August 2023
Published: 30 August 2023

Keywords: Metoclopramide, Visible Spectroscopy, Validation Parameters, Sodium periodate, DNPH

ABSTRACT

A simple colorimetric method has been described for the determination of Metoclopramide hydrochloride by sodium periodate in the presence of 2,4-dinitrophenylhydrazine in an acidic medium and measurement of absorbance of orange color formed at 495nm. Parameters affecting the reaction were studied and conditions were optimized. Linear calibration graph was obtained on a concentration range of 5-35µg/ml of Metoclopramide hydrochloride. Limit of detection was found to be 3.91µg/ml and the Limit of quantification was found to be 11.857. The regression value was found to be 0.992. The method was successfully applied for the determination of Metoclopramide hydrochloride in pharmaceutical preparations. No interference was observed from common pharmaceutical adjuvants. Hence this method can be successfully applied for the determination of Metoclopramide hydrochloride in both pure and pharmaceutical formulations.

INTRODUCTION

Pharmaceutical analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. The term “Pharmaceutical Analysis” is otherwise called Quantitative pharmaceutical chemistry. Pharmaceutical analysis includes both quantitative and qualitative analysis of drugs and pharmaceutical substances starting from bulk drugs to the finished dosage forms.

The importance of analytical chemistry lies in the fact that it is the foundation pillar of the entire procedure of drug discovery, isolation, standardization and quality control. The basic importance of pharmaceutical analysis is its utility in the standardization and quality control of medicines and drug substances to ensure the quality and stability of the final product.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modifications of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

The functional group present in organic drugs determines the way of analyzing them because they are responsible for the properties of substances, determining the identification reactions and the method of quantitative determination of drugs. Knowing the reactions for detecting functionalgroups, one can easily analyze any organic drug with a complicated structure.

Dinitrophenyl Hydrazine (DNPH, Brady’s reagent)

It is a chemical compound $C_6H_3(NO_2)_2NHNH_2$. It is a red to orange solid, It is a substituted hydrazine and it is often used to qualitatively test for carbonyl groups associated with aldehydesand ketones. The positive test is signaled by a yellow, orange or red precipitate (Dinitrophenylhydrazone).

Validation may be defined as a process involving confirmation or establishing by laboratory studies that a method/ system/analyst gives accurate and reproducible results for intended analytical application in a proven and established range.

The wide use of MCP has prompted the development of several analytical methods for its determination in pharmaceuticals when present alone or in combination with other drugs and include HPLC, LC-MS, fluorimetry, spectrophotometry etc. Some analytical techniques have also been reported for the determination of MCP in biological matrices. Despite the availability of sophisticated and sensitive instruments for the assay of MCP some visible spectrophotometric methods based on redox reactions, ion pair complex formation and diazo coupling reaction and using reagents such as sodium vanadate, ammonium metavanadate, folin-ciocalteu, 1,2-naphthaquinone-4-sulfonate and p-dimethyl amino cinnamaldehyde, charge transfer complex formation have also been reported. Most of the reported visible spectrophotometric methods suffer from one or other disadvantage like poor sensitivity, drastic experimental conditions like heating, strict pH control liquid-liquid extraction step, use of organic solvent, narrow linear range etc. Visible spectrophotometric method those could be used to determine MCP in tablets is thus considered.

Metoclopramide is 4-amino-5-chloro-N-[2-diethylaminoethyl]-2methoxybenzamidehydrochloride monohydrate. It is a D_2 receptor antagonist, $5HT_3$receptor antagonist and $5HT_4$ receptor agonist. Due to the blockade of D_2 receptors, dopamine loses its inhibitory control over Acetylcholine release from primary cholinergic neuron in myenteric plexus. It is mainly used as an anti-emetic drug.

MATERIALS AND METHODS

Preparation of standard stock solution

Standard metoclopramide 100 mg was weighed accurately and transferred into a 100 volumetric flask. It was dissolved properly in distilled water and made up to the mark 10ml of this solution was transferred to a 100ml volumetric flask and made up to 100ml with distilled water to get a concentration of 100 µg/ml.

2,4- dinitro phenyl hydrazine (2,4-depth) 0.08% (w/w) (bradys reagent)

0.08g of 2,4-DNPH reagent was accurately weighed and transferred into 100 ml volumetric flask, dissolved in 2 ml H_2SO_4 and diluted to 100ml mark with distilled water.

Sodium periodate 4% (w/v)

A 4%w/v sodium per iodate solution was prepared by dissolving 4g in 100ml distilled water.

5m sodium hydroxide solution

20g of sodium hydroxide was dissolved in 100ml distilled water.

Determination of λ max

Aliquots of the standard stock solution in distilled water were transferred into a series of 10 ml volumetric flasks. To each 1.5ml 0.08% 2,4-DNPH and 1.5ml 4% sodium periodate were added, which were made alkaline by adding 1ml 5M Sodium hydroxide. The contents of the flask were shaken thoroughly and made up with distilled water. The resulting orange-coloured solution was scanned over visible range of 400-800nm against the reagent blank. An overlay spectrum of the drug was drawn out and a wavelength at which the drug showed maximum absorbance

Determination of linearity

Aliquots of the standard stock solution in water, having a final concentration in the range of 5- 35µg/ml were transferred into a series of 10 volumetric flasks. To each 1.5ml of 0.08% 2,4- DNPH and 1.5ml of 4% sodium periodate solutions were added, which were made into alkaline by adding 1ml each of 5M sodium hydroxide. The contents of the flask were shaken thoroughly and made up with water. The absorbance of the resulting solution was measured at 495nm against the reagent blank and the calibration curve was drawn by plotting the absorbance against concentration.

OPTIMIZATION OF EXPERIMENTAL CONDITION

Effect of volumes of reagent and acid catalyst

Different volumes of prepared 2,4-DNPH reagent from 0.5-3.5ml and in parallel with the volume of sulphuric acid from 1-3ml were studied with the mentioned procedure. Optimum color intensity and reproducible (λ max) values were obtained with 1.5ml of reagent and 2ml of acid catalyst.

Effect of volume and concentration of base

It was observed that maximum intensity, better resolution with sharp peak and reproducible (λ max) values were obtained with 1ml of 5M sodium hydroxide. With less concentration of base, the colour of the blank remains dark, and higher concentration showed fluctuations in λ max values.

Effect of time on analysis

The orange-colored complex produced was stable only for about 15 minutes. So the absorbance should be measured immediately after preparation of the sample.

Analysis of tablet formulation

10 tablets were accurately weighed and triturate thoroughly to get a fine powder. The powder equivalent to 10mg of metoclopramide was weighed and transferred into 100ml volumetric flask. The contents of the flask were dissolved in distilled water with aid of 10 minutes. The solution was filtered through Whatman filter paper no.41 and volumes were made up to 100ml with distilled water.

From the resultant solution, further dilutions were prepared with freshly prepared 0.08% DNPH,4% sodium periodate,5M NaOH and distilled water to get final concentration of metoclopramide. The absorbance was measured against the reagent blank at 495nm.[25]

METHOD VALIDATION

The method was validated with respect to linearity, range, LOD, LOQ, Accuracy, Precision Robustness and Ruggedness.

Linearity

Linearity was checked by preparing standard solution at different six concentrations ranging from 5-35µg of MCP. The calibration curve was plotted between the difference in absorbance and concentration and optical parameters.

LOD and LOQ

In this study, LOD and LOQ were based on the standard deviation of response (σ) and the slopeof the corresponding curve using the following equations

$$LOD = 3.3\sigma/SLOQ= 10\sigma/S$$

Accuracy

The accuracy of the proposed method was determined by calculating the recoveries of Metoclopramide by the standard addition method. It was determined by preparing solutions of different concentrations at 80 %, 100 %, and 120 % in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. The amount of MCP was estimated by applying the obtained values to the regression line equation.

Precision

The precision of the method was determined by performing Interday variation, intraday variation and repeatability studies and expressed in the forms of % RSD. In interday variation, the absorbances of working standard solutions of MCP were measured on three consecutive days. In intraday variation, the absorbance was measured three times a day. In the repeatability study, six determinations of the fixed concentration of both acidic and basic solutions of the drug were analyzed separately.

Ruggedness

Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In the present study, the determination of MCP was carried out by different analysts. The percentage purity of the drug was determined and % RSD was calculated.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In this method, it was performed by changing temperature (room temperature and at 21º C). The percentage purity of the drug was determined.

RESULTS AND DISCUSSION

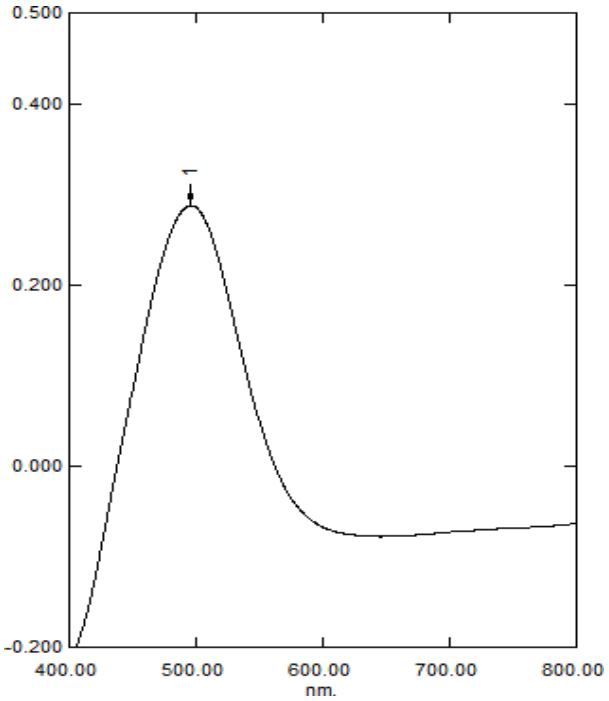

Fig. 1: λ max of MCP at 495nm

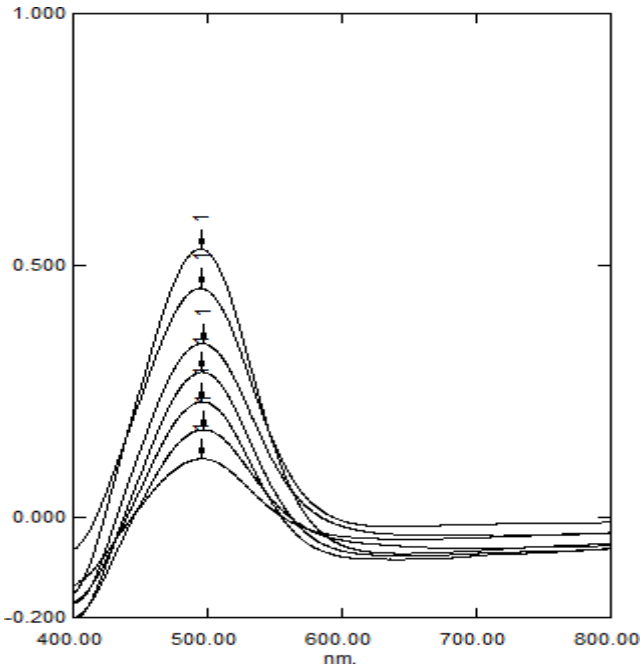

Fig. 2: Overlay spectrum of MCP at 495nm

Table 1: Linearity of MCP

Sl. No	Concentration of MCP	Absorbance at 495nm
1	5	0.116
2	10	0.165
3	15	0.233
4	20	0.289
5	25	0.367
6	30	0.450
7	35	0.538

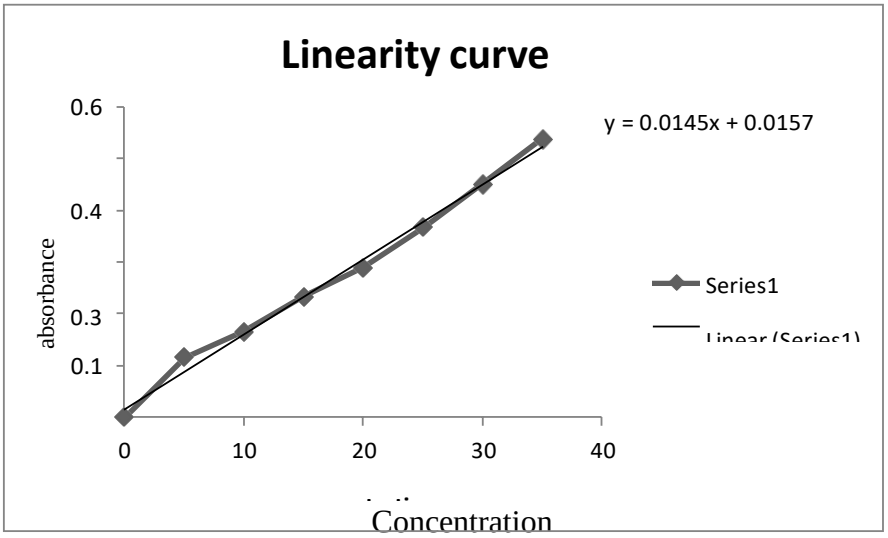

Fig. 3: Linearity of MCP

Table 2: Optical characteristics of MCP

PARAMETERS	VALUES
Color	Orange
λ max	495 nm
Linearity range	5 - 35 µg/ml
Regression Equation	Y=0.014x + 0.015
Slope	0.014
Intercept	0.015
Correlation coefficient	0.992
LOD	3.91 µg/ml
LOQ	11.859 µg/ml
Molar Absorptivity (mean)	1340.75 L/mol/cm

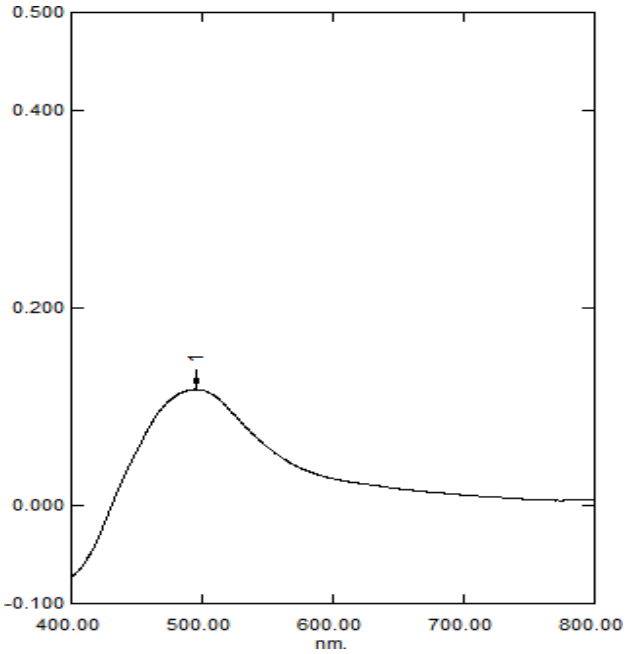

Fig. 4: Spectra of tablet formulation

Table 3: Analysis of marketed formulation

Formulation	Label claim	Amount Estimated(mg)	% Amount estimated	SD	%RSD
MCP (PERINORM)	10 mg	10.28 mg	102.8%	0.911	0.893

Table 4: Recovery study

Standard Drug Added(%)	Amount of Pure Drug Added (µg/ml)	Amount of Formulation Added (µg/ml)	% Recovery	Standard Deviation	%RSD
80	16	20	99.77%	0.165	0.165
100	20	20	99.62%	0.440	0.447
120	24	20	99.59%	0.605	0.607

Table 5: Precision data

Particulars	Fortified Amount(µg/ml)	AmountFound (µg/ml)	SD	%RSD
Repeatability	20	20.66	0.193	0.934
Reproducibility	20	20.88	0.081	0.383

Table 6: Ruggedness

Component	Label Claim	ANALYST 1			ANALYST 2		
		% Amount Estimated	SD	% RSD	% Amount Estimated	SD	% RSD
MCP	10mg	99.96	0.76	0.760	99.89	0.608	0.608

Table 7: Robustness

Component	Label Claim	At 21˚C			At room temperature		
		% Amount Estimated	SD	% RSD	% Amount Estimated	SD	% RSD
MCP	10mg	99.24	0.640	0.644	99.82	0.575	0.576

CONCLUSION

A simple accurate, precise, and economical spectrophotometric method has been developed for the quantitative estimation of MCP in bulk and pharmaceutical formulation. The present work complied with our initial research objective and successfully demonstrated the applicability of the spectrophotometric method for the analysis of MCP.

The promising result from the present research reveals the need of further extensive study of the drug using the tremendous potential of various analytical instruments. The development of innovative methodologies will unquestionably expand future research capabilities in terms of shorter run times, and highly rugged and reproducible methods with high accuracy.

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