Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF METOCLOPRAMIDE HYDROCHLORIDE IN BULK AND TABLET FORMULATION

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ABSTRACT

The present research work discusses the development of UV-spectroscopic method for estimation of metoclopramide. Simple, specific, accurate and cost effective spectroscopic method has been developed for estimation of metoclopramide hydrochloride in bulk as well as formulation. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 272 nm. The validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The method shows high sensitivity with linearity in the range of 2-20 µg/ml. all calibration curves shows a linear relationship between the absorbance and concentration with coefficient of correlation 0.998. The regression of curve was $Y = 0.33x + 0.022$. The precision of method was found to be good. The percentage recovery was found to be 100% ± SD. The optimized showed good reproducibility and recovery with standard deviation < 1 % and percent relative standard deviation less than 2 %, standard error in case of recovery studies are satisfactorily low and allow estimation metoclopramide in concentration range employed for this purpose in the assay of bulk drug and tablets. The sample solution was stable upto 36 hours. The proposed method will be suitable for analysis of metoclopramide in bulk as well as pharmaceutical formulations in quality control purpose. It is thus concluded that the proposed method is new, simple, cost-effective, safe, accurate, precise and environmental friendly.

Keywords: Metoclopramide, Accuracy, Sensitive, ICH guidelines

INTRODUCTION

Metoclopramide Hydrochloride **(MCP)** is used as Dopamine receptor antagonist; antiemetic. Chemically it is 4-amino-5-chloro-*N*-[2-(diethylamino) ethyl]-2-methoxybenzamide hydrochloride (**Fig 1**). MCP is used as Dopamine receptor antagonist; antiemetic. It is available as white or almost white, crystalline powder or crystals, which is very soluble in water, freely soluble in alcohol, sparingly soluble in methylene chloride. This antiemetic, chemically related to the procainamide, acts predominantly as a *dopamine antagonist*[1-2]. Literature survey reveals the estimation of MCP in pharmaceutical formulations by HPLC[3-8] and spectrophometric methods[9-12]. The aim of present work was to develop simple, sensitive specific spectrophotometric method for detection of MCP in bulk as well as pharmaceutical formulation.
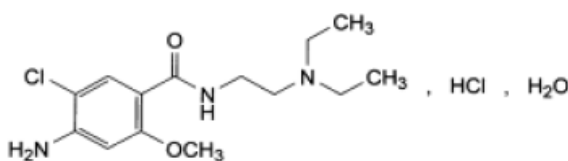

Fig. 1: Structure of Metoclopramide Hydrochloride

MATERIALS AND METHODS

Materials

Metoclopramide hydrochloride was supplied by Ajanta Pharmaceuticals Ltd (Mumbai, India). All other chemicals were purchased from local vendor and used without further purification. For preparation of analytical reagents and solutions Milli-Q Pore water was used.

Instrumentation

Drug concentrations in various tests were determined spectrophotometrically (SL-164 Double beam UV spectrophotometer, *Elico*, India.) at 272 n using 1 cm quartz match cells. For dilutions various micropipettes of volumes 10-100 µl (Gene Pete Co.) was used. All weighing operations were done on *Schimadzu* Digital Analytical Balance (Japan).

METHODS

Preparation of standard stock solution

100 mg of MCP was weighed accurately and transferred to 100 ml of calibrated volumetric flask. 50 ml of previously prepared and standardized 0.1 N HCl was transferred to same flask and swirled for solublization. Volume was made upto 100 ml mark by 0.1 N to obtain solution of 1mg/ml concentration. This solution was used as standard stock solution. From this solution 10 ml was withdrawn and diluted to 100 ml with 0.1 N HCl, (100 µg/ml). This solution was used as working standard solution. For calibration curve dilutions were made from 2-20 µg/ml.

Preparation of sample solution

The average weight of tablets was determined by weighing 20 tablets and powdered. Tablet powder weight equivalent to 20 mg of MCP was weighed and transferred to 100 ml volumetric flask. About 50 ml of 0.1 N HCl was added and sonicated for 15-20 min for compete dissolution of drug. Volume was made upto 100 ml with 0.1 N HCl and mixed. Above solution was filtered through Whatmann filter paper No. 41 and further diluted to obtain solution of 10µg/ml. Six replicates of analysis were carried out.

Method validation

Various method of analysis of MCP in bulk and pharmaceutical formulations (marketed and developed) was carried out as ICH guidelines Q2(R) [13-17].

Measurement of absorbance and calibration curve

The absorbance of solutions containing 10µg/ml was determined in UV range 200-800 nm using 0.1 N HCl as blank. The λ_{max} was found to be 272 nm. At this wavelength maximum, calibration curve was drawn by plotting graph between absorbance and concentration.

Linearity

The aliquots of concentrations ranging from 1-30 µg/ml were prepared in triplicate, but linearity was found to be between 2-20 µg/ml. the linearity was calculated by the least square regression method.

Precision and accuracy

Precision is degree of repeatability of an analytical method under normal operation conditions. The precision and accuracy were determined with standard quality control samples prepared in triplicate at different concentration levels covering the entire linearity range. The precision of assay was determined by repeatability (intraday), intermediate precision (inter-day) and reported as %RSD for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assay on three different days and the results are documented as the standard deviation and %RSD. Accuracy is the percentage of analyte recovered by assay from known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve using following equation:

LOD = 3.3 σ/ S

Where σ is standard deviation of Y-intercept and S is slope of calibration curve.

The limit of quantification (LOQ) is defined as the lowest concentration of calibration curve that can be measured with an acceptable accuracy, precision and variability. The value of LOQ was determined using following equation:

LOQ = = 10 σ/ S

Recovery study

Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations, as employed in the linearity studies, was used. To study the accuracy, precision and reproducibility of the proposed method and dosage forms, recovery experiments were carried out using the standard addition method. These studies were performed by the addition of known amounts of pure MCP to the pre-analyzed tablet formulation and the mixtures were analyzed using the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

Stability studies

The stability of RIS in 0.1N HCL solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 4 and 25°C for 3 days.

RESULTS AND DISCUSSION

The development of a simple, rapid, sensitive and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. In the start of the method development for this drug, different solvents were tested such as water, methanol, 0.1N HCl, 0.1N NaOH and Phosphate buffer (pH7.4). Due to greater solubility and reproducible readings of maximum absorbance, 0.1 N HCl was taken under consideration for further work.

MCP is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region. It was found to be that only one peak was observed in this method at the wavelength of 272 nm.

Calibration curves

Calibration curve data were constructed in the range of the expected concentrations of 2 µg/mL to 20 µg/mL. *Beer's* law was obeyed over this concentration range. The regression equation was found to be Y = 0.33x + 0.022. The correlation coefficient (r) of the standard curve was found to be greater than 0.998. The stock solutions and working standards were made in 0.1N HCL. Linearity range and calibration curve is presented in **Table 1** and **Figure 2**.

Table 1: Linearity study of MCP in 0.1 N HCl at 272 nm

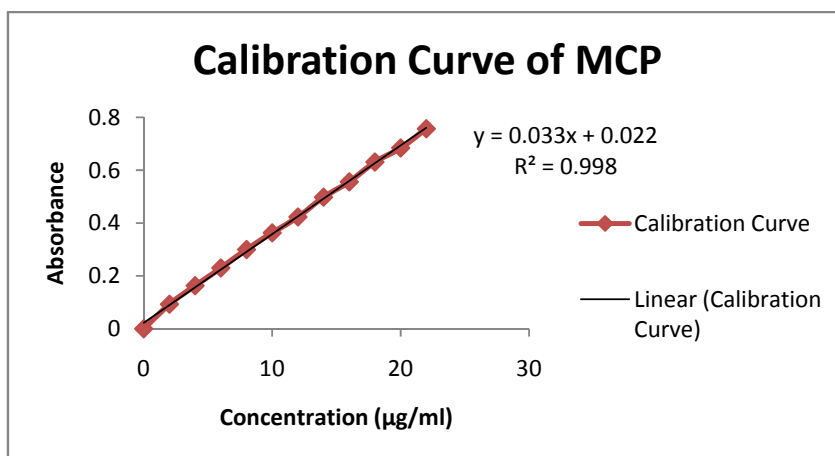| Sl. No.	Concentration (µg/ml)	Absorbance
1.	2	0.093
2.	4	0.183
3.	6	0.230
4.	8	0.300
5.	10	0.363
6.	12	0.423
7.	14	0.498

Fig. 2: Calibration curve of MCP

Analytical method validation

The data was statistically validated by means of least square regression method. The detection and quantization limits were found to be **1.98** and **2.26**. The mean percentage drug estimated was 100.57 indicating the accuracy of the proposed analytical method (**Table 2**).

Table 2: Result of pharmaceutical formulation analysis

Parameter	Marketed Sample	Formulated Tablet	Formulated Capsule
Label claim (mg)	20	20	20
Found (mg)	20.11	20.17	20.27
Drug content	100.55	100.85	101.35
±SD	0.130	0.220	0.410
%COV	0.090	0.156	0.189
SE	0.189	0.170	0.221

Table 3: Results of recovery studies

Formulation	Label claim (mg)	Amount (mg/ml)		% Recovery ±SD	%COV
		Taken	Added		
Marketed	20	5	2	100.03 ±0.12	0.211
		10	4	100.12 ±0.37	0.203
		15	6	101.33 ±0.55	0.321
Tablet	20	5	2	101.23 ±0.27	0.322
		10	4	99.26 ±0.45	0.431
		15	6	101.24 ±0.17	0.377
Capsule	20	5	2	100.61 ±0.45	0.473
		10	4	101.26 ±0.61	0.457
		15	6	101.33 ±0.55	0.624

% Recovery is mean of three estimations; SD: Standard deviation; COV: Coefficient of variance

Mean standard deviation and standard error for MCP was found to be 0.253 and 0.193 respectively. The low values of these statistical parameters validated the method. The value of mean percentage recovery was 100.16 (**Table 3**). This fact, together with satisfactory low values of statistical parameters (**Table 4**), further validated the method. There was no interference of excipients in the estimation. The proposed method can be successfully employed in the routine analysis of metoclopramide containing dosage forms.

Table 4: Validation data of UV method for MCP

Linearity Range (µg/ml)	Precision				LOD (µg/ml)	LOQ (µg/ml)	Stability (days)
	Intra-day (%COV)	Inter-day (%COV)					
		Day 1[a]	Day 2[a]	Day 3[a]			
2-22	0.190	0.225	0.277	0.251	1.98	2.26	3

[a] Mean of five determinations, **COV:** coefficient of variance, **LOD:** limit of detection. **LOQ:** limit of quantization.

CONCLUSION

The developed spectrophotometric method was simple, sensitive, and specific, for the detection of MCP in bulk & pharmaceutical formulation. It could be precisely quantify and LOD was found to be 1.98 and the limit of quantification to be 2.26. All the calibration curves shows a linear relationship between the absorbance and concentration and coefficient correlation was higher than 0.99. Precision of the method was found to good against the label claim of 20 mg. The percentage recovery was found to be satisfactory and the sample solution was stable for up to 3 days. The proposed method will be suitable for the analysis of MCP in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENT

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