Development and Validation of UV-Spectroscopic Method for Assay of Metoclopramide Hydrochloride in Bulk and Injectable Dosage form

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Abstract

This research work aimed to develop a simple, specific, accurate and cost effective spectroscopic method for the estimation of Metoclopramide hydrochloride in bulk as well as parentral formulation. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 270 nm. The validation was performed according to ICH guidelines. The proposed method showed high sensitivity with linearity in the range of 5µg/ml to 30µg/ml. All calibration curves showed a linear relationship between the absorbance and concentration with correlation-coefficient R^2=0.9998. The precision of the method was found to be good. The recovery percentages were found to be 101.77%, 100.27% and 101.23%. The method was found to be robust as the RSD was 1.75%, and precise with RSD values of (0.869%, 1.17% and 0.925%) for the repeatability, intraday precision and interday precision, respectively. The LOD and LOQ are found to be 0.34µg/ml and 1.031µg/ml, respectively. The proposed method will be suitable for analysis of Metoclopramide in bulk as well as parentral pharmaceutical formulations for quality control purpose. We concluded that this method is simple, cost-effective, safe, accurate, precise and environmental friendly.

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1. Introduction

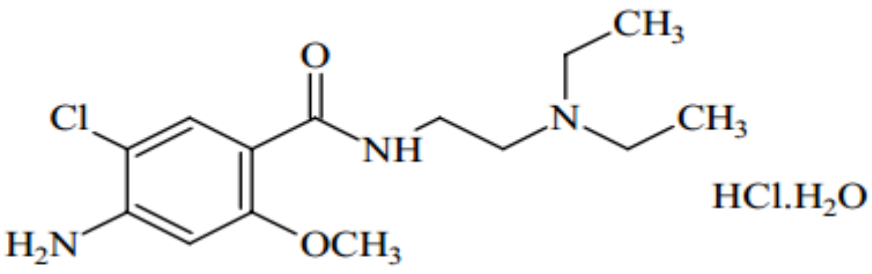
Metoclopramide-hydrochloride (MCPH) is a white, odorless, crystalline powder (melting point about 185 C°), very soluble in water, alcohol and partially insoluble in ether. Chemically (MCPH) is: 4-amino-5-chloro-N-(2-diethylaminoethyl)-2 methoxybenzamidemonohydrochloride monohydrate [1].

Metoclopramide was used as a treatment of nausea and vomiting in association with migraine and severe headache. The drug was used for the control of sickness due to radiation therapy and chemotherapy, and for the prevention and treatment of post operative nausea and vomiting. Metoclopramide also has a gastrointestinal pro-kinetic effect through cholinergic stimulation [2].

The most common adverse effects of Metoclopramide involve the central nervous system [3].

Liquid chromatography (HPLC) is the official method for assay of MCP in BP and USP [4] [5]; further, literature survey revealed HPLC [6] [7] and spectrophometric methods for estimation of MCP in pharmaceutical formulations [8] [9].

Direct UV-spectroscopy was the basis of this research work; considering the pharmacophoric groups in the molecule of Metoclopramide which absorb in UV range. The wave length of 270 nm recorded to have the maximum absorbance in the using 0.1 M HCl as solvent. On the contrary to common pharmaceutical dosage forms such as tablets and suspensions, injectable dosage forms are almost excipients-free; consequently fewer interference will be encountered.



$C_{14}H_{22}ClN_3O_2.HCl.H_2O$

$MW = 354.3\ g.mol^{-1}$

Fig. 1: Structure of Metclopromide Hydrochloride [1].

2. Materials and methods

2.1. Materials

2.1.1. Apparatus

Pipettes (Marienfeld Germany) Tol±0.05, measuring cylinders (polylab) ± 0.5, beakers (ISOlab Germany), burettes (ISOlab Germany)± 0.05, volumetric flasks (ISOlab Germany) ±0.4, boro 3.3, conical flasks (ISOlab Germany), spatula.

2.1.2. Instruments

UV/Visible spectro-photomerty from PG Instruments Limited Company, Model: T80+ with serial number: 23-1885-01-0065 and manufacture date: 05/2014.

Sensitive balance from B0ECO company, Model: BAS32 with serial number: 405400.

2.1.3. Chemicals

MCP standard (PharmaLand Company, assay99.9).

Hydrochloric acid 0.1M (Chemlab NA assay 37%).

Distilled water.

MCP injections brand (A) (L.B.S Laboratory LTD).

MCP injection brand (B) (Sanofi Winthrop industry).

2.2. Methods

2.2.1. Preparation of Hydrochloric acid (HCl) 1M, 0.1M and 0.01M

From stock solution of 37% hydrochloric acid, volumes of 83.3, 8.3 and 10 ml were added to 1000 ml volumetric flasks to make 1M, 0.1M and 0.01M HCl, respectively, and the volumes were completed by distilled water.

2.2.2. Preparation of standard stock solution

Ten miligrams of standard Metoclopramide (MCP) was weighed and transferred to 100 ml volumetric flask then the volume was completed to 100 ml by 0.1M HCl to make 100µg/ml.

2.2.3. Optimization

Serial dilutions of standard (5, 10, 15, 20, 25, and 30µg/ml) were prepared from working MCP standard solution by taking different volumes (1.25, 2.5, 3.75, 5, 6.25 and 7.5ml) from stock solution and transfer them to 25 ml volumetric flask then the volume was completed by three different concentrations of HCl (0.01, 0.1, and 1 M). The absorbance was measured at 270 nm then calibration curve and R^2 regression was obtained.

2.2.4. Determination of maximum wave length

Concentration of 20µg/ml was prepared from standard MCP solution and scanned by UV visible spectrophotometer in range of 200-400 nm using 0.1 M HCl as blank then the maximum wave length (λ-max) was determined.

2.2.5. Method Validation

2.2.5.1. Linearity

Serial dilutions (5, 10, 15, 20, 25, and 30µg/ml) of MCP standard were prepared from working MCP standard solution, the absorbance was measured at 270 nm using 0.1M HCL as blank then calibration curve and R^2 regression was obtained.

2.2.5.2. Precision

a. Repeatability

Six different preparations of the same concentration (20µg/ml) were prepared from working MCP standard solution then absorbance was measured at 270 nm by using 0.1M HCL as blank and RSD was calculated.

b. Intraday Precision

Three different preparations of the same concentration (20µg/ml) were prepared by different operators from MCP standard solution then the absorbance of each was measured at time zero, 2h and 4h after the preparation at 270 nm using 0.1M HCL as blank and RSD was obtained.

c. Interday Precision

Three different preparations of the same concentration (20µg/ml) of MCP were prepared from working standard solution by different operators in three consequence days then the absorbance was measured at 270 nm using 0.1M HCl as blank and RSD was calculated.

2.2.5.3. Accuracy

Three preparations from each concentration 20µg/ml (100%), 24µg/ml (120%) and 16µg/ml (80%) were prepared from working MCP standard solution then the absorbance was measured and RSD was calculated.

2.2.5.4. Robustness

Concentration of 20µg/ml was prepared from working MCP standard solution. The absorbance was measured at different wave length 270, 270.1, 269.9, 272 and 268 using 0.1M HCL as blank then RSD was calculated.

2.2.5.5. Sensitivity

To determine the sensitivity, the **LOD** and the **LOQ** were calculated from linearity's curve.

3. Results

3.1. Determination of the maximum wave length

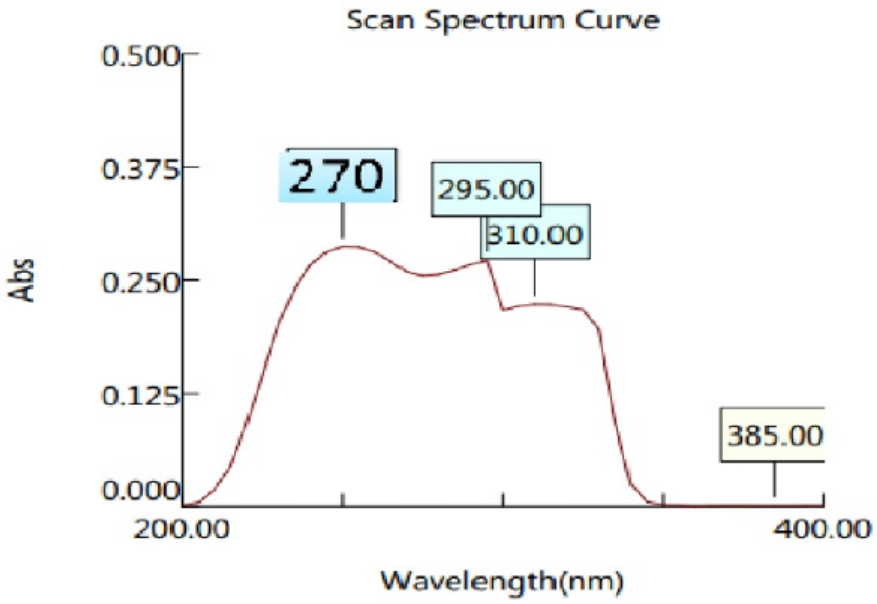


Fig. 2: Scan spectrum curve of MCP standard solution.

3.2. The method optimization

Table 1: The optimization study of MCP in different HCl concentrations.

Concentration (µg /ml)	HCl (0.01M)	HCl (0.1M)	HCl (1M)
5	0.210	0.174	0.057
10	0.427	0.354	0.143
15	0.666	0.522	0.207
20	0.859	0.695	0.276
25	1.069	0.870	0.326
30	1.305	1.035	0.391
R^2	**0.9993**	**0.9998**	**0.9943**

3.3. Linearity

Table 2: Linearity study of MCP standard solution using 0.1M HCl and wave length 270 nm

Concentration (µg/ml)	Absorbance
5	0.174
10	0.354
15	0.522
20	0.695
25	0.870
30	1.035

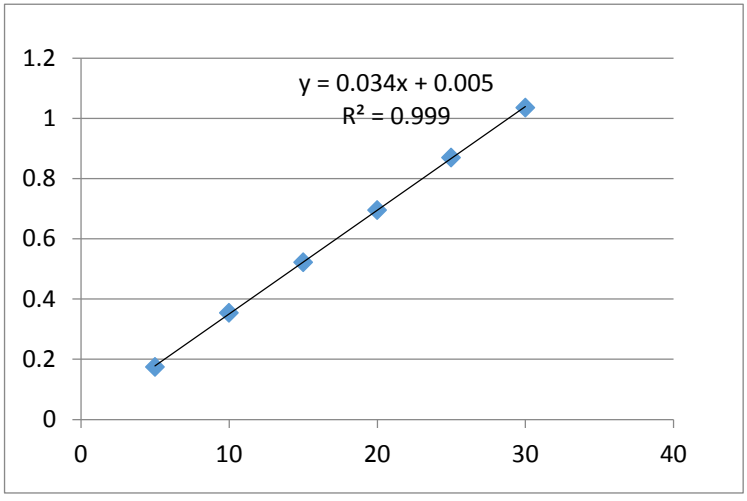


Fig. 3: The calibration curve of MCP standard solution using 0.1M HCl and wave length 270 nm.

3.4. Sensitivity

It was determined by the limit of detection (LOD) and the limit of quantification (LOQ) values, which were calculated from the linearity data, as the following: LOD = 0.340548µg/ml, LOQ = 1.031963 µg/m

3.5. Precision

3.5.1. Repeatability

Table 3: The repeatability study of MCP standard solution using 0.1M HCl and wave length 270 nm

Concentration (µg /ml)	Absorbance at 270nm
20	0.681
20	0.692
20	0.698
20	0.696
20	0.695
20	0.692
RSD = 0.869%	

3.5.2. Intraday precision

Table 4: Intraday precision study of MCP standard solution using 0.1M HCl and wave length 270nm

Preparations (20 µg /ml)	Absorbance At time Zero	Absorbance after 2 hours	Absorbance after 4 hours
1	0.681	0.685	0.682
2	0.692	0.691	0.674
3	0.698	0.695	0.691
RSD = 1.17%			

3.5.3. Interday Precision

Table 5: Interday precision study of MCP standard solution (20 µg /ml) using 0.1M HCl and wave length 270nm

Days/Operators	Operator 1	Operator 2	Operator 3
Day 1	0.633	0.647	0.649
Day 2	0.647	0.641	0.651
Day 3	0.651	0.647	0.647
RSD = 0.925%			

3.6. Robustness

Table 6: Robustness study of MCP standard solution of concentration 20 µg/ml using 0.1M HCl

Wave Length(nm)	Absorbance
270	0.668
270.1	0.680
269.9	0.688
272	0.687
268	0.661
RSD = 1.75%	

3.7. Accuracy

A. Recovery Percent

Table 7: Recovery percent study of MCP standard solution using 0.1M HCl at wave length 270 nm

Concentrations (µg /ml)	Absorbance (nm)	Recovery Percent
20 µg /ml (100%)	0.636	99.68%
	0.639	100.156%
	0.639	100.156%
24 µg /ml (120%)	0.78	99.529%
	0.762	99.529%
	0.753	98.354%
16 µg /ml (80%)	0.511	100.11%
	0.513	100.509%
	0.519	101.68%

B. Standard Addition

i. The sample results

Table 8: The sample results for standard addition using 0.1M HCl and wave length 270 nm.

	Preparations	Absorbance (nm)
Concentration (10 µg /ml)	1	0.362
	2	0.362
	3	0.362

ii. The standard results

Table 9: The standard results for standard addition using 0.1M HCl and wave length 270 nm

Concentration (µg /ml)	Absorbance (nm)
5	0.169
10	0.359
15	0.504

iii. The Mixture Results

Table 10: The mixture results for standard addition using 0.1M HCl and wave length 270 nm

Concentration (µg /ml)	Absorbance (nm)
15	0.534
20	0.722
25	0.873

4. Discussion

This method has been introduced for the analysis of MCP since the official method (HPLC) is not usually available everywhere.

MCP is soluble in many solvents, i.e. water, HCl, alcohol and chloroform and partially insoluble in ether [10], in our study HCl was selected as it is available and easy to prepare, also it give an excellent absorbance results.

The selected concentration of the HCl is 0.1 M, determined by the optimization step, which confirmed that the absorbance values are very low in the case of concentration 1M; between 0.057 and 0.391, with R^2 value 0.9993, on other hand the absorbance values are too high and exceed 1 in the case of 0.001M HCl; between 0.210 and 1.305 with R^2 value 0.9943. However in the case of 0.1M HCl the absorbance values are good, between 0.174 and 1.035, with R^2 value 0.9998.

Firstly the concentrations used are (2, 4, 6, 8 and 10 µg /ml), but they give low absorbance values between 0.079 and 0.387 with R^2 value 0.998, so they are substituted with (5, 10, 15, 20, 25 and 30 µg /ml) which give excellent absorbance with R^2 value 0.999.

The maximum wave length of absorption of MCP was found to be 270nm, which is a good, because it is not detecting most of the excepients that may present in the formulation, while it is 272 nm in both

Wamorkar et.al [11] and Anton Smith et.al. Studies [12]. Also we found that there is a linear relationship between the concentration and the absorbance, as the R^2 was 0.999 (≈1) that indicate the linearity of the method, while in Wamorkar et.al study the R^2 = 0.998 [11] and was 0.999 in Anton smith et.al [12].

In the accuracy measurement using the standard addition method, the RSD% value was 0.894%, which is less than 2 and the add recovery percent of standard addition results were 101.77%, 100.27% and 101.23% that means this method is accurate. While in Wamorkar and his workers the recovery percent was found %100% ± SD, the SD < 1 [11].

The repeatability test; the RSD% value was 0.869% for six preparations for the same concentration prepared in the same conditions, and that mean the method is repeatable. While in the intraday precision study the RSD% value was 1.17% for absorbance values of three concentrations prepared by different operators and the absorbance values of these preparations were determined in the same conditions at time zero, after two hours and after three hours, this indicate the method is stable within the same day. The RSD% value was 0.925% for absorbance values of three concentrations prepared by the same operator but in different days; that mean the method is precise among different days, while Musab et.al study show relative standard deviations of (1.2, 0.478 and 1.32) % for repeatability, intraday and inter day precision respectively [2].

The RSD% was 1.75% when different wave lengths were used (270, 270.1, 269.9, 272 and 268 nm); that mean the method will not be affected by the small changes, for example the errors in the monochrometer or operator, so this method is considered as a robust method.

The minimum concentrations that can be detected and quantified are 0.340548µg/ml and 1.031963µg/ml, respectively. While the other studies used diazotization coupling reaction to form color dye of MCP found that; the limit of detection (LOD) and limit of quantification (LOQ) are 0.016 ppm, and 0.054 ppm respectively [1]. Moreover, A. N. Alshirifi and M. H. Abbas study showed that; limit of detection (LOD) and limit of quantification (LOQ) values with 0.012 ppm, and 0.043 ppm, respectively [1].

In this study we used two brands of MCP hydrochloride injections, as they have different formulas, to make sure that this method is effective with the different brands.

Brand (B) is a “preservative formula”, which contain “Benzyl alcohol”. This may be responsible from the increasing in the absorbance values when compare it’s values with the absorbance of the same concentrations of the standard, as reported that benzyl alcohol can cause interference in the absorbance of MCP [13], and so the content percent found to be 108.6%. Also this brand is “Antioxidant-free”, so it is in a “Light-protected containers”.

Brand (A) “preservative-free formula”, so it is not containing “Benzyl alcohol”. So the absorbance values was a near to the standard’s absorbance values. Also this brand contain “Antioxidant” so it is in normal containers, this antioxidant was not affect the absorbance, as the content% is 100.1%, since the range reported in both BP and USP is (90%-110%) [4] [5].

5. Conclusion

The new developed method was valid because it was found to be easy, specific, linear, sensitive, accurate, precise, stable and robust method.

6. Acknowledgement

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References

[1] A. N. Alshirifi and M. H. Abbas, “New Spectrophotometric method for the determination of Metoclopramide Hydrochloride in pharmaceutical preparations based on coupling with doxycycline hycylate,” *Int. J. Chem. Sci*, vol. 13, no. 3, pp. 1093–1108, 2015.

[2] Musab M.Ibrahim, Mohamed Musa Ahmed, Yasser Mohammedien Al-Amien, “International Journal of Innovative Pharmaceutical Sciences and Research Method for analysis of Metoclopramide Hydrochloride in bulk and pharmaceutical preparations,” *Int. J. Innov. Pharm. Sci. Res.*, vol. 3, no. 1558, pp. 1558–1568, 2015.

[3] Reglan, “REGLAN Injection (metoclopramide injection , USP),” 2010.

[4] *British pharmacopeia* 2013. Volume II. London: The Stationary Office Ltd, 2013, monograph of Metoclpramide.

[5] *United States Pharmacopiea* 2013. Volume 2.

[6] Budavari S. Eds. The Merck Index, 13th edition, Merck and Co. Inc. White House Station, NJ, 2001, 1094.

[7] Takahashi H, et al. "Determination of Metoclopramide and its Glucuronide and Sulphate Conjugates in Human Biological Fluids (Plasma, Urine and Bile) By Ion-Pair High-Performance Liquid Chromatography". J. Chromatogr 1987; 419:243.

[8] Albani F. et al. "Sensitive Liquid Chromatography–Tandem Mass Spectrometry Method for the Determination of Metoclopramide in Human Plasma". Biomed Chromatogr 1987; 2: 135.

[9] Patel MM, Patel CM, Patel SA. "Visible Spectrophotometric Method for the Estimation of Metoclopramide Hydrochloride in Tablets". Indian J Pharm. Sci. 2006; 68:3 397-9.

[10] S. A. Mohammed and K. M. Al-abbasi, "Spectrophotometric Determination of Metoclopramide Hydrochloride in Pharmaceutical Preparations Using Diazotization Reaction," vol. 22, no. 3, pp. 76–88, 2011.

[11] V. Wamorkar, S. Y. Manjunath, and M. M. Varma, "Development and Validation of spectroscopic method for determination of Metoclopramide Hydrochloride in Bulk and Tablet formulation Calibration Curve of MCP," *Int. J. Pharm. Pharm. Sci.*, vol. 3, no. 3, pp. 10–13, 2011.

[12] A. Thangamani, A. A. Smith, and B. Vedhapriya, "Develoment of analytical method for Metoclopramide using UV-Spectrophotometry," *Int. J. Pharm. Chem. Biol. Sci.*, vol. 4, no. 3, pp. 551–555, 2014.

[13] G. H. B.-S. AB, "Use of benzyl alcohol as a shipping and storage solution for chromatography media," 2012.