Validation and application of RP-HPLC method for the quantification of metoclopramide hydrochloride in oral formulations prepared for IVIVC studies

Ahmad Khan[*1]**, Syed Baqir Shyum Naqvi**[1]**, Muhammad Harris Shoaib**[1]**, Rabia Ismail Yousaf**[1]**, Jallat Khan**[2]**, Muhammad Hanif**[1] **and Asadullah Madni**[3]

[1]Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Pakistan
[2]Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[3]Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Abstract: The objective of this study is to develop sensitive and cost effective reverse phase high performance liquid chromatographic method for the estimation of Metoclopramide Hydrochloride in oral solid dosage formulations. A reverse chromatographic method was used with the mobile phase of Acetonitrile, 20m M Potassium dihydrogen phosphate buffer solution (pH 3 adjusted with orthophosphoric acid) in the ratio of 40:60. The column used was Waters C18 3.9×300mm µBondapak (RP). The flow rate of the mobile phase was 2ml/minute. The detector was set at the wavelength of 275nm.This method showed good sensitivity. The linearity was also found to be excellent (γ^2=0.997) in the range of 5-75 µg/ml. No interfering peaks were observed at the retention time of Metoclopramide Hydrochloride when both placebo and blank samples were injected (Retention time =1.93min). The parameters such as specificity, linearity, range, accuracy, precision, system suitability, solution stability, detection and quantification limits were evaluated to validate this method. This method can effectively be used for quantitative analysis of Metoclopramide hydrochloride tablet formulations because of its specificity, accuracy and convenience of use.

Keywords: Metoclopramide Hydrochloride, chromatography, placebo study, system suitability, UV- detection.

INTRODUCTION

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-*N*-(2-diethylamino-ethyl) benzamide (fig. 1), is dopamine receptor antagonist, mainly used as antiemetic, a stimulant of the upper gastrointestinal motility and used for management of gastrointestinal disorders and reflux. It is also used as a preventive medicine for cancer chemotherapy -induced emesis at higher doses (Hassan and Enein, 1990, Shidhhaye and Malke, 2009, Venkateshwaran and King, 1995, Indian Pharmacopoeia, 2007, Phamacists., 1989).

Due to the applications of Metoclopramide hydrochloride in clinical and experimental medicines, it has a lot of literature on its determination in biological fluids and dosage forms. Both the United States Pharmacopoeia (The United States Pharmacopoeia, 2000) and the British Pharmacopoeia (British Pharmacopoeia, 1998) recommend a non-aqueous acid-base titration with potentiometric detection of the end-point for the evaluation of the raw material of metoclopramide from its dosage forms. USP recommends HPLC methods and the BP describes spectrophotometric methods of analysis.
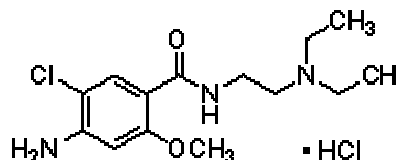

Fig. 1: Chemical structure of metoclopramide hydrochloride

The analytical methods reported for the determination of Metoclopramide in dosage forms and in biological fluids are various chromatographic procedures. However often due to consumption of more organic solvents, having long run time, utilize solid phase extraction which is time consuming and may lead to a lower drug recovery when they are used. This study describes a specific, sensitive and cost effective rapid assay with short run time of 5 minutes for determination of metoclopramide hydrochloride in its formulations.

MATERIALS AND METHODS

Chemical and reagents

Metoclopramide Hydrochloride was gifted by Indus Parma (Pvt.) Limited Pakistan, Avicel PH-102 (FMC-Corporation, USA), Lactose (Dow Chemical, USA), Crosspovidone, (ISP Technologies, Inc. Wayne, NJ), Magnesium Stearate (Dow Chemical, USA), HPMC

Corresponding author: e-mail: ahmadkingsk@yahoo.com

4000cps (Dow chemical, USP Type 2208), Sodium Hydroxide, (Merk KGaA, Germany), Potassium Dihydrogen Phosphate (Merk KGaA, Germany), Methanol (HPLC/Spectro., Tedia, USA), Acetonitrile, Orthophosphoric acid (Merk, KGaA, Germany). All chemicals used were of analytical grade.

Equipments
Analytical balance (Sartorius: CP224S, Serial No.14411659, Germany), Liquid Chromatograph (LC-10AT, Shimadzu, Japan), Communications Bus Module (CBM-102, Shimadzu, Japan), Waters C18 3.9×300mm µBondapak (RP) Column, pH meter (370 pH meter, Jenway, Europe), Syringe (Hamilton Company, Reno, Nevada), Swinney filter (stainless steel, 13 mm filter, Millipore Corporation, Billerica, USA), Membrane Disc Filters (MILLIPORE, 0.45µm pore size, Millipore Corporation, Billerica, USA), Sonicator (LC20H), Vortex (FSA Lab., England).

Method
Instrumentation and chromatographic conditions
High performance liquid chromatographic system (LC-10A vp, Shimadzu, Japan), consisting of pump (LC-10A vp, Shimadzu, Japan), Communications Bus Module (CBM-102, Shimadzu, Japan), UV–VIS detector (LC-10A vp, Shimadzu, Japan), computer (Pentium 4) and printer (HP LaserJet 2015).

For the separation, Waters C18 3.9×300mm µBondapak (RP) Column was used and the chromatograms were recorded in the Class-GC10 software (2.00). Mobile phase was prepared from acetonitrile and buffer (Potassium Dihydrogen Phosphate, pH adjusted to 3 with orthophosphoric acid) in the ratio of 40:60, filtered through 0.45µm and degassed. The flow rate was kept at 2ml/ minute. The sample of 60µl was injected and detected at 275nm and the separation was carried out at ambient temperature.

Preparation of solutions
1. Buffer preparation
3mM buffer solution of Potassium Dihydrogen Phosphate was prepared and the pH was adjusted to 3.0 with orthophosphoric acid).

2. Mobile phase preparation
Acetonitrile and buffer in the ratio of 40:60 were mixed to prepare the mobile phase. It was filtered under vacuum and finally sonicated for 15minutes before use.

3. Standard preparation
Stock solution of strength 0.05 % of metoclopramide hydrochloride was prepared in mobile phase and solution of concentrations 25 µg/ml, 37.5 µg/ml, 50 µg/ml and 62.5, 75 µg/ml were prepared in the same solvent system.

Preparation of formulations of metoclopramide hydrochloride
All formulations were prepared containing Metoclopramide Hydrochloride (10mg) by direct compression method. Different ranges of excipients were used each for Immediate release (X1) HPMC K4M (4%-6%), (X2) MCC PH-1O2 (25%-40%), (X3) Lactose DC (25%-60%), for Intermediate release(X1) HPMC K4M (10%-20%), (X2) MCC PH-1O2 (30%-40%), (X3) Lactose DC (30%-40%), for slow release HPMC K4M (40%-60%), (X2) MCC PH-1O2 (30%-60%), (X3) Magnesium Stearate (1%-5%), and excipients were weighed accurately mixed geometrically and finally compressed at target weight 120mg per tablet. Central Composite design was used separate for immediate, intermediate and slow release formulations from which one formulation was optimized each from immediate intermediate and slow release formulations. Among all formulation three optimized formulations were selected which passed all the quality control tests, their content uniformity was performed by this proposed method and the results were listed (table 7).

4. Sample Preparation
20 tablets from each formulation containing 10mg of drug were selected randomly and accurately weighed .Each tablet was ground to fine powder in mortar and pestle and the powder was diluted to 0.005% in mobile phase.

Validation of the method
Buss *et al*, in 1990 reported HPLC Method for the estimation of Metoclopramide Hydrochloride in blood (Buss *et al*., 1990).This reported method was modified and validated for the determination of drug in tablet dosage formulations.

Parameters studied for analytical assay validation were linearity, selectivity, accuracy, precision, system suitability, solution stability, LOD, and LOQ under the guidelines of ICH Q2B (International Conference on the Harmonization, 1996).

1. Specificity
Specificity is the parameter that helps to discriminate between the analyte and other components present in the sample. Therefore in order to detect interference of solvents and formulation excipients used in tablets specificity was evaluated by comparing the chromatograms of six replicate injections of each placebo with the standard metoclopramide hydrochloride (Lister, 2005).

2. Linearity
It is the characteristic that shows linear relationship between the range of concentrations and the detector response (Lister, 2005). For assessing linearity 50 µg/ml was kept as centre point i.e. (100%) two higher and two

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lower concentrations were prepared from stock solution (0.05%) and found the linearity (Lister, 2005) (table 1).

Fig. 3 represents the concentration range analysed by the proposed method which was then subjected to regression analysis for the determination of coefficient of correlation ($r^2 = 0.997$).

3. Accuracy
The closeness in the nominal and the actual results obtained is known as accuracy. Accuracy was evaluated by spiked placebo recovery method. (Hassan and Enein, 1990). Sample solutions with 25μg/ml, 50μg/ml, and 75μg/ml concentrations were prepared and spiked with placebo solution. The results of recovery are expressed as percentage recovery and obtained by comparing the ratio of drug samples with standard.

4. Precision
Inter-day precision was assessed by analysis of three selected concentrations for three consecutive days whereas intra-day precision was carried out by the analysis of the standard solution in triplicate throughout the linearity range (Shubhangee *et al.*, 2010).

5. System Suitability
System suitability parameters are useful to evaluate the adequacy of system performance. The typical system suitability parameters were determined by injecting five replicates of 50 μg/ml concentrations and the peak area, reproducibility, capacity factor, tailing factor, theoretical plates and resolution were recorded by Class-GC10 software (version 2.00) (Hassan and Enein, 1990)

6. Solution Stability
Solution Stability is essential for the solutions that will be analysed for their quantification. Evaluation of shelf life was carried out by keeping the sample at ambient temperature for 12 hours. Same assessment was made at -15°C to -20°C for 7days.The results were within the acceptance limit (Lister, 2005) (table 3).

7. Limit of quantization (LOQ)
Lowest concentration of the analyte that can be quantitated with acceptable degree of certainty (Lister, 2005). Since the standard curve was investigated from 2.5μg/ml to 75μg/ml, the former was considered as LOQ. The analyte peak (response) was clearly identifiable, discrete, and reproducible as per FDA guidance (FDA, 2001).

8. Limit of detection (LOD)
Limit of detection shows the smallest amount of analyte in a given method that may be detected but not necessarily quantitated (Lister, 2005). For the determination of LOD of this method 5μg/ml of standard solution was injected five times and was set as LOD since

the concentrations analysed in method were high enough to be easily detected.

RESULT

For the quantitative estimation of metoclopramide hydrochloride in tablet dosage forms a reversed phase method was proposed as a suitable method. At 275nm metoclopramide showed good sensitivity and selectivity. Method was developed on Waters C18 3.9×300mm μBondapak (RP) Column which provided efficient separation at ambient temperature. The mobile phase composition, acetonitrile: buffer (40:60) yielded baseline resolved peaks with an excellent resolution. For the sake of method validation guidelines by the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (*ICH*) Q2B (1996) (International Conference on the Harmonization, 1996) were followed which recommends the accomplishment of accuracy ,precision,linearity,specificity, solution stability, system suitability, LOD and LOQ.

DISCUSSIONS

1. Specificity
No interference from the tablets excipients was detected as indicated by the combined chromatogram of blank, placebo and standard (fig. 2) (Lister, 2005)]. The retention time for placebo and standard chromatograms were 1.51min and 1.93 min respectively.
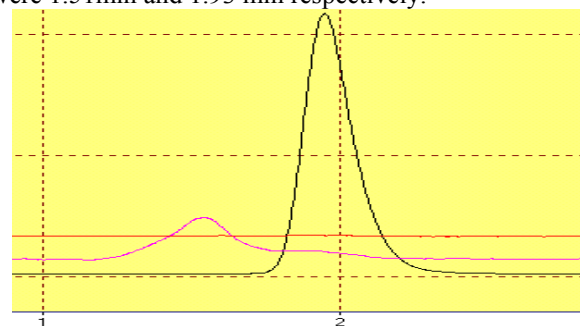

Fig. 2: Combined chromatograms of blank (40:60ratio of acetonitrile and buffer) zero peak, placebo and % solution of metoclopramide hydrochloride by using the validated method, flow rate was 2ml/min, injection volume: 60μl, UV detection: 275nm.The run time was 5 minutes for all injections.

2. Linearity
For linearity determination five concentrations (International Conference on the Harmonization, 1996) which were equally spaced throughout the range of the method, (5μg/ml, 25μg/ml, 37.5μg/ml, 50μg/ml, 62.5μg/ml, 75μg/ml respectively), were used. Linear regression by the least square method was then applied for the determination of coefficient of correlation value ($\gamma^2=0.997$) (table 1) indicated excellent linearity obtained.

Similar results of correlation coefficient (fig. 3) were obtained by some workers (Ganesh *et al*., 2009, Fronza *et al*., 2006, Rehman *et al*., 2010).

Table 1: The linearity characteristics of RP-HPLC method

Concentration (%)	Concentration (ug/ml)
50	25
75	37.5
100	50
125	62.5
150	75
y-intercept	-8266
Correlation coefficient,r	0.997

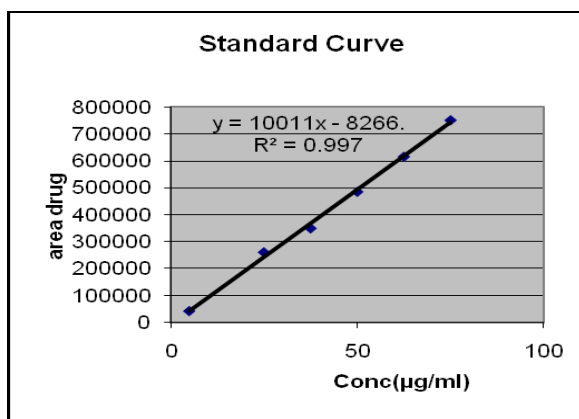**Standard Curve**

$y = 10011x - 8266.$
$R^2 = 0.997$

Fig. 3: Linearity curve

3. System Suitability

To evaluate the resolution and reproducibility of the method by the system for the analysis of the drug to be performed the system suitability tests (fig. 4) were also carried out. System suitability parameters tests were, %RSD (Relative Standard Deviation) of retention time, peak area, tailing factor, and theoretical plates were within the range, as listed in tables 2 & 4.
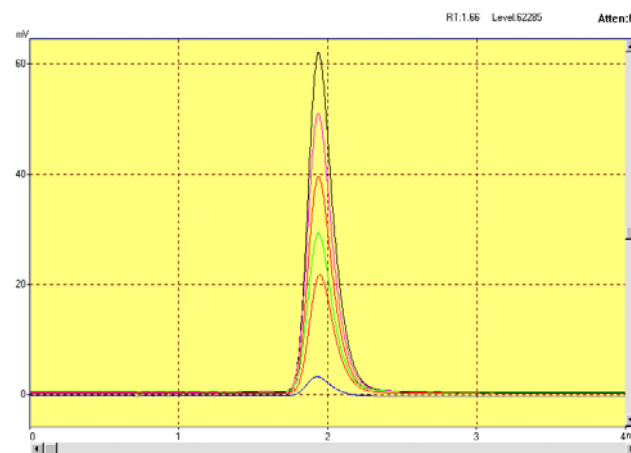

Fig. 4: Chromatograms of the system suitability.

4. Accuracy

The results obtained from the replicate injections of three selected concentrations 25μg/ml, 50μg/ml, and 75μg/ml provided the evidence for the method accuracy. The mean percent recovery for each concentration is presented in table 5.

5. Precision

Intra-day and interday precision determination was carried out by the analysis of the standard solution by injecting five injections each of 25 μg/m, 50 μg/ml and 75 μg/ml on the same day and on three consecutive days respectively and mean % RSD was calculated for the injections, intraday (1.12%, limit less than 2%) and inter day (1.14%, limit less than 2%) as listed in table 6.

Content uniformity of the three optimized formulations

The potencies found in immediate release, intermediate release and slow release optimized formulations were calculated by the formula (Tansuva *et al*., 2010) and are listed in table 7.

Table 2: System suitability parameters

No. of Injections	Standard Solution 60μL	Retention Time	Area	Tailing Factor	Theoretical Plates
1	50 μg/ml	1.93	485288	1.38	3125
2	50 μg/ml	1.94	490707	1.38	3165
3	50 μg/ml	1.93	492849	1.40	3142
4	50 μg/ml	1.93	480789	1.38	3154
5	50 μg/ml	1.93	492512	1.38	3102

Table 3: Standard and sample solutions.

Condition	Theoretical conc. μg/ml	*Results Obtained μg/ml	% Recovery	% RSD
Ambient temperature (for 12hrs)	50	49.98	99.87	0.16
-15 Ċ to -20 Ċ (for 7 days)	50	48.76	99.98	0.21

*Results are mean of 6 values.

Table 4: Results of system suitability

Parameter	Mean	RSD (%)	Limits
Retention time	1.93	0.23	-
Area	488429	1.07	Less than 2
Tailing factor	1.384	0.6	Less than 2
Theoretical plates	3156.6	1.02	-

Table 5: Optimized immediate release formulation

S. No.	Concentration	Found(µg/ml) Mean ± SD n=5	Recovery (%) n=5
1	25 µg/ml	25.05±0.04	100.05
2	50 µg/ml	49.92±0.06	99.84
3	75 µg/ml	74.97±0.02	99.96

Table 6: Precision of the proposed method

S. No.	Concentration	Interday Mean n =5	Interday RSD %	Intraday (3 days) Mean n =5	Intraday (3 days) RSD %
1	25 µg/ml	25.07	1.04	24.76	0.89
2	50 µg/ml	49.93	1.24	50.04	1.21
3	75 µg/ml	74.79	1.16	74.92	1.27

Table 7: Drug content determination present in three different formulations of Metoclopramide HCl by this proposed method.

Formulation	Measured amount(mg) n=5 (Mean±SD)	Added amount(mg) (Actual amount per tablet)	Potency (%)
Immediate Release	9.987±0.41	10	99.87
Intermediate Release	9.965±0.22	10	99.65
Slow Release	9.945±0.35	10	99.45

% Potency = measured amount ×100/claimed amount (Tansuva *et al.*, 2010)

CONCLUSION

The validation data shows that the proposed high performance liquid chromatographic method for measuring metoclopramide hydrochloride in pharmaceutical dosage formulations is simple, accurate, and reliable and has excellent linearity and precision characteristics. The short run time, reproducible, in expansive, less volume of injections this method therefore can be recommended for the routine analysis.

ACKNOWLEDGEMENTS

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