Validation and application of high performance liquid chromatographic method for the estimation of metoclopramide hydrochloride in plasma

Ahmad Khan*[1], Jallat Khan[2], Muhammad Irfan[3], Syed Baqir Shyum Naqvi[4], Gul Majid Khan[1], Muhammad Harris Shoaib[5], Rabia Ismail Yousaf[5] and Amjad Khan[6]

[1]Department of Pharmacy, Quid-i-Azam University, Islamabad, Pakistan
[2]Department of Chemistry, Islamia University, Bahawalpur, Pakistan
[3]Deparment of Pharmacy, GC University, Faisalabad, Pakistan
[4]Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[5]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[6]Department of Biotechnology, Quid-i-Azam University, Islamabad, Pakistan

Abstract: The objective of this study was validation of a reverse phase HPLC method for the estimation of metoclopramide HCl in plasma already validated for determination of metoclopramide HCl in tablets dosage form. A reverse chromatographic method was used for estimation of metoclopramide HCl with the mobile phase of acetonitrile, 20mM potassium dihydrogen phosphate buffer solution (pH 3.0 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 validated in plasma and was found to be linear, with correlation coefficient (R^2), value of 0.9988, in the range of 48 ng/ml-0.25ng/ml. The method modified was accurate, precise, sensitive and showed good stability results. The % RSD of the retention time and peak area of metoclopramide HCl was 0.19% and 1.44% respectively. All the parameters such as specificity, linearity, range, accuracy, precision, system suitability, solution stability, detection and quantification limits were evaluated to validate this method and were found within the acceptance limits. The method can be effectively used for estimation of metoclopramide HCl in plasma.

Keywords: Metoclopramide HCl, HPLC, validation, pharmacokinetics, plasma.

INTRODUCTION

Metoclopramide is used in the treatment of motility disorder of upper GI (Gastrointestinal Tract) tract, particularly delayed gastric emptying, gastro-esophageal reflux, nausea and vomiting from causes other than motion sickness, migraine, particularly to increase the absorption of analgesics, Diagnostic radiology to speed the passage of contrast media into the small bowel, in the treatment of non-specific upper gastrointestinal symptoms, including gastritis, flatulence and heartburn, diagnostic investigation of pituitary function, particularly prolactin release, specifically in high doses for treatment of cytotoxic-induced nausea, defective lactation, tardive dyskinesia (Martindale's, 2009). Metoclopramide is the only drug for the gastroparesis treatment approved by FDA to date (Smith and Ferris, 2003).After oral dosing Metoclopramide is almost completely absorbed, and achieves peak plasma concentrations within 0.5-2.0 hours in fasted healthy volunteers (Bateman *et al*., 1980). The half-life of metoclopramide is 3-5 hours in healthy male volunteers. Oral metoclopramide HCl tablet is administered in a dose of 10-15 mg four times a day so that to maintain affective concentration in the body (Kasim *et al*., 2003).

This method validation was done for the estimation of Metoclopramide HCl in human plasma (KHAN *et al*., 2015). This study describes a specific, sensitive, and fast sample preparation assay for the determination of metoclopramide HCl in human plasma and can successfully be applied to the Pharmacokinetic study of metoclopramide HCl in man.

MATERIAL AND METHODS

Materials

Cannula with stopper (Master IV Catheter, Korea), Sterile BD syringes (Becton Dickinson), Heparinized Centrifuged Tubes, Cotton Wool (Locally Manufactured), Ethanol (Merk, Germany), Microlitter Pipette (Vocal, England), Refrigerator (LG Electronics, Korea), Acetonitrile (Merk, KGaA, Germany), Orthophosphoric Acid (Merk, KGaA, Germany), Methanol (Tedia, USA, HPLC grade), Drug free human plasma was obtained from Modern Hospital Karachi, Distilled Water, always prepared freshly by distillation, metoclopramide HCl tablets (Three optimized formulations i.e. Immediate, F2 intermediate, F10 and slow, F18), Reference Brand Maxolon® (Purchased from local market).

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

Apparatus

Liquid Chromatograph (LC-10AT VP, Shimadzu, Japan), UV Detector (SPD-10A, 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 (Millipore, Millipore Corporation, Billerica, USA), Membrane Disc Filters (Millipore, 0.45µm pore size, Millipore Corporation, Billerica, USA), Sonicator (Clifton Ultrasonic bath, England), Vortex (Y2K 04053, Whirlmixer, England), Filtration Assembly (Sartorius, Germany).

Softwares used

Kinetica® 4.4.1 PK/PD and WinNonlin® IVIVC Toolkit 1.0.

Methods

The method reported by Buss *et al*, 1990 was modified and validated for metoclopramide HCl in plasma (Buss *et al*., 1990).

Table 1: Results of system suitability (n=6)

Parameter	Mean	RSD (%)	Limits
Retention time	1.7	0.19	-
Peak Area	85449	1.44	Less than 2
Tailing factor	1.567	0.77	Less than 2
Theoretical plates	2678	1.02	-

Preparation of mobile phase

A 3.0mM buffer solution of Potassium Dihydrogen Phosphate was prepared and the pH was adjusted to 3.0 with orthophosphoric acid. 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 15 minutes before use.

Preparation of stock solution in mobile phase

Stock solution of strength 0.05% of metoclopramide hydrochloride was prepared in mobile phase.

Preparation of stock solution in plasma

Stock solution of strength 0.05% of metoclopramide hydrochloride was prepared in plasma.

Extraction of metoclopramide HCl from plasma (back extraction)

Samples of Plasma containing drug (1.0ml) were taken in small tubes, 100µl, 1.0M NaOH was added, Then 5ml from 90:10 of chlorobutane and acetonitrile mixture was added into it, These tubes were vortexed for 1 minute and Centrifuged at 2500g for 5 mints, Supernatants were collected and transferred to new tubes, Then 300µl 0.1M HCl was added to these tubes, These tubes were again vortexed for 1 minute and centrifuged for at 2500rpm for 5 minutes, 80µl aliquot was separated from each tube and 60µl was injected into the HPLC System for analysis (Buss *et al*., 1990).

Assay method

Stock solution (0.05%) of metoclopramide HCl was prepared in mobile phase, Different concentrations of metoclopramide HCl stock solution were (25µg/ml, 37.5µg/ml, 50µg/ml and 62.5, 75µg/ml), The concentration ranges from 48ng/ml-0.5ng/ml were selected for the determination of lowest and highest quantity of the drug in the plasma, Different concentration of metoclopramide HCl in mobile phase and plasma were run in the HPLC for drug recovery to validate the method.

Validation of HPLC method in human plasma

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

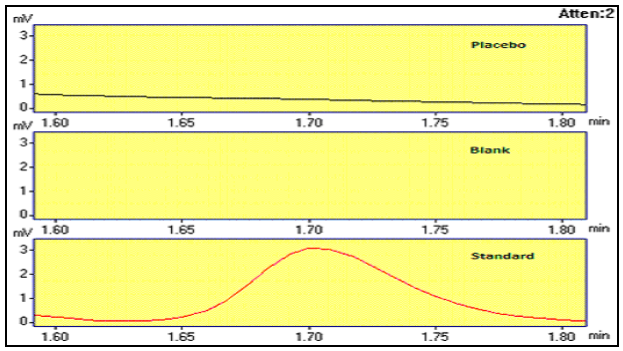

Fig. 1: specificity of Metoclopramide hcl in plasma (n=6)

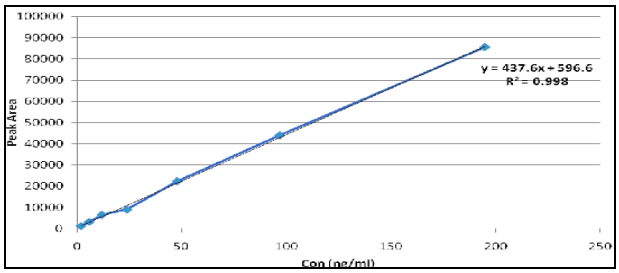

Fig. 2: Linearity of metoclopramide HCl in plasma

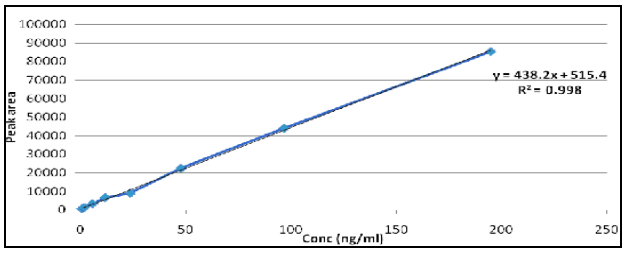

Fig. 3: Linearity with LOD and LOQ of metoclopramide HCl in plasma

Specificity

Specificity is the parameter that helps to discriminate between the analyte and other components present in the

Table 2: Intraday accuracy

Concentration ng/ml						
	12		24		48	
	Conc	Found	Conc	Found	Conc	Found
1	12	11.70	24	24.63	48	47.74
2	12	11.69	24	23.57	48	47.73
3	12	11.68	24	23.59	48	47.77
4	12	11.72	24	23.58	48	47.76
5	12	11.71	23	23.62	48	47.75
Mean	12	11.70	24	23.79	48	47.75
±SD	0.08		0.13		1.95	
Recovery (%)	97.50		98.33		98.43	

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 HCl (Lister, 2005).

Linearity

It is the characteristic that shows linear relationship between the range of concentrations and the detector response (Lister, 2005). For assessing linearity (n=5) concentration ranging from 48ng/ml-0.25ng/ml were prepared by serial dilution method and the values of the coefficient of correlation (R^2) were calculated for each data set.

Table 3: Intraday precision

Concentration ng/ml			
	12	24	48
1	11.89	23.35	48.03
2	11.96	23.33	47.96
3	11.94	23.34	48.03
4	12.02	23.37	47.95
5	11.96	23.36	48.06
Mean	11.95	23.35	47.98
RSD%	0.39	0.39	0.06

Accuracy

Sample solutions with 12, 24 and 48ng/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. The SD, recovery % and % accuracy were then calculated.

Precision

Inter-day precision was assessed by analysis of three selected concentrations (12, 24 and 48ng/ml) for three consecutive days whereas intra-day precision was carried out by the analysis of the standard solution in triplicate throughout the linearity range. The %CV was calculated.

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 48ng/ml concentrations and the peak area, reproducibility, capacity factor, tailing factor, theoretical plates and resolution were recorded by Class-GC10 software (version 2.00) The % RSD was calculated.

Table 4: Interday precision

Concentration ng/ml			
Day	12	24	48
1.00	11.46	24.02	47.65
	11.47	23.71	48.05
	11.44	23.74	47.69
	11.45	23.75	47.77
	11.45	23.70	47.65
2.00	11.47	23.73	47.56
	11.45	24.69	47.59
	11.44	23.71	47.63
	11.44	23.75	47.64
	11.45	23.73	47.55
3.00	11.44	24.01	48.02
	11.46	23.88	47.68
	11.43	23.74	47.67
	11.44	24.00	47.65
	11.47	23.69	47.58
Mean	11.45	23.84	47.69
RSD %	0.11	1.08	0.31

Solution stability

Evaluation of shelf life was carried out by keeping the sample (48ng/ml-0.25ng/ml) at ambient temperature for 12 hours. Same assessment was made at -15C^o to -20C^o for 7 days. The % CV was calculated (Lister, 2005).

Limit of quantization (LOQ)

Since the standard curve was investigated from 48ng/ml-0.25ng/ml, 0.5ng/ml was considered as LOQ. The analyte peak (response) was clearly identifiable, discrete, and reproducible as per FDA guidance.

Limit of detection (LOD)

For the determination of LOD of this method 0.25ng/ml of standard solution was injected five times and was set as LOD since the concentrations analysed in this were high enough to be easily detected.

Table 5: Standard and sample solutions stability in plasma

Conc 48ng/ml			
	Fresh Sample	Ambient Temperature (For 12hrs)	(-15°C to -20°C) (For 7 days)
	ng/ml	ng/ml	ng/ml
Sample 1	47.96	47.48	47.95
Sample 2	47.95	47.88	47.84
Sample 3	47.99	47.87	47.86
Sample 4	47.65	47.96	47.87
Sample 5	48.02	47.82	47.76
Sample 6	48.05	47.93	47.85
Mean	47.93	47.82	47.85
% Recovery	99.86	99.63	97.69
% RSD	0.03	0.36	0.12

RESULTS

Charts and chromatograms of HPLC method validation was given in figs. 1-3and tables 1-5. The calibration curve for metoclopramide HCl in the concentration range of 48ng/ml-0.25ng/ml is shown in fig. 2. System suitability results in plasma are presented in table 1. Table 2-4 show the results accuracy and precision of the method. Fig. 3 shows the results of linearity with LOD and LOQ in plasma. table 5 shows the results of the stability of the method.

DISCUSSION

HPLC method validation in plasma

Several analytical method have been reported for the quantification of metoclopramide HCl in biological fluids (Javanbakht *et al*., 2009, Supriya *et al*., 2008, Bryson *et al*., 1984, Vlase *et al*., 2006, Jordan *et al*., 1999, Maurich *et al*., 1994, Aqeel *et al*., 1989, Beckett *et al*., 1987, Hamamoto *et al*., 2013, Inamadugu *et al*., 2010, Yan *et al*., 2010) some of these are complicated and more time consuming (Horiuchi *et al*., 2006, Nieder and Jaeger, 1987, Riggs *et al*., 1994). In this study the modified and validated method by Buss *et al* 1990 for the content assay of Metoclopramide HCl, was also validated for plasma following the ICH (Q2B) guidelines (Harmonization, 1996). No interfering peaks appeared on the chromatograms for both the blank and sample at the retention time of metoclopramide HCl, which confirms the method specificity (fig. 1). This method was found to be linear, with the coefficient of correlation (R^2=0.9988) and showed linearity from 48 ng/ml-0.25ng/ml (fig. 2), accurate, precise, sensitive and showed good stability results. The system suitability test was performed by collecting the data from replicated injections of the standard solution of metoclopramide hydrochloride (table 1). The % RSD of the retention time and peak area of metoclopramide hydrochloride from six (6) consecutive injections of the standard solution was (0.19%) and (1.44 %) respectively. Based on the USP tangent calculations the mean ±RSD theoretical plate count for metoclopramide hydrochloride peak obtained from six consecutive injections of the system suitability solution was 2678±1.02 % (table 2). The accuracy ±SD on three different concentrations 48, 24, 12ng/ml was found to be 47.75±1.95, 23.79±0.13, 11.70±0.08 ng/ml with absolute analytical recovery (%) values of 98.43, 98.33 and 97.50 respectively (table 2). Measurement of closeness of the values to each other for a no of measurements under the same analytical conditions is termed as precision (CDER, 1994). The interday precision ±RSD% on three different concentrations 48, 24, 12ng/ml was calculated to be 47.69±0.39, 23.84±1.08 and 11.45±0.011 respectively (table 4). While intraday precision ±RSD% on the same concentrations was 47.98±0.06, 23.35±0.39 and 11.95±0.39 respectively (table 3). The lower limit of Quantification (LOQ) was 0.5ng/ml and Lower Limit of Detection (LOD) was 0.25ng/ml. fig. 3shows the result of linearity with LOD and LOQ. Nieder and Jaeger in 2005 applied an HPLC method for quantization of metoclopramide in human plasma and urine. Both the metoclopramide and the internal standard were extracted from alkalinized substrate into diethyl ether and back extracted into dilute acid. Their sample preparation technique was liquid extraction. The chromatographic conditions included the use of a CN column and UV detection at 312nm. The LOQ in plasma and mobile phase was 0.5ng/ml and 50ng/ml respectively. The method was found selective, accurate and precise (Nieder and Jaeger, 1987).

The stability was also determined for six samples of 48 ng/ml at ambient temperatures and -15°C to -20°C (for seven days). The % recovery ±RSD was found to be 99.63±0.36 and 97.69±0.12 respectively (table 5) (Riggs *et al*., 1994).Jordan *et al* in 1999 developed a rapid, reproducible, sensitive reverse phase HPLC quantitative procedure for metoclopramide in serum. In this method single step extraction procedure for metoclopramide and disopyramide (IS) from alkalinized serum into the benzene using a reverse phase C-8 system. The mobile phase used was 11: 22: 66, methanol: Acetonitrile: pH 3.7 acetate buffer. The detection was made at 268 nm. This method showed successful results in clinical pharmacokinetic studies involving IV oral administration

of metoclopramide to cancer patients receiving highly emetogeniccis-diamminedichoroplatinum (Jordan *et al*., 1999).

CONCLUSION

This proposed method has significant sensitivity and linearity for the determination of pharmacokinetics of metoclopramide HCl in human healthy volunteers. Due to accuracy specificity and linearity, this method can be used in analysis of metoclopramide HCl in plasma of human volunteers for future IVIVC studies. The method was effectively used for estimation of Metoclopramide HCl in human plasma.

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