



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Rapid Determination of Metoclopramide Level in Human Plasma by LC-MS/MS Assay

Rajaa F Hussein, Syed N Alvi, and Muhammad M Hammami*

Clinical Studies and Empirical Ethics Department, King Faisal Specialist Hospital & Research Center, MBC-03, P.O. Box 3354, Riyadh 11211, Saudi Arabia

ABSTRACT

A rapid liquid chromatographic tandem mass spectrometry (LC-MS/MS) assay for the measurement of metoclopramide level in human plasma was developed and validated. One ml plasma samples containing metoclopramide and 0.25 µg of loratadine as internal standard (IS) were extracted with 5 ml *tert*-butyl methyl ether and reconstituted in 80 µl of acetonitrile. Analysis was performed on reversed phase Atlantis dC₁₈ column using a mobile phase of 0.4% formic acid (pH=3.0 ± 0.05) and acetonitrile (20:80, v:v) delivered at a flow rate of 0.25 ml/minute. Analytes were quantified multiple reaction monitoring in positive ion mode with transition mass to charge ratio (m/z) of 299.8→226.9 and 383.4→337.2 for metoclopramide and IS, respectively. Retention times of metoclopramide and IS were around 1.4 and 2.1 minutes, respectively. No significant matrix effect was observed on metoclopramide and IS peaks. Detection limit of metoclopramide in plasma was 0.3 ng/ml. Relationship between metoclopramide level and peak area ratio of metoclopramide / IS was linear ($R^2 \geq 0.9964$) in the range of 0.5–100 ng/ml and inter-day coefficient of variations (CV) and absolute bias were $\leq 12.0\%$ and $\leq 6.0\%$, respectively. Mean extraction recoveries for metoclopramide and the IS were 91% and 88%, respectively. The method was applied to assess stability of metoclopramide under various conditions generally encountered in the clinical laboratory. Stability of metoclopramide was $\geq 94\%$ and $\geq 95\%$ after 24 hours at room temperature or 48 hours at -20 °C, respectively, in processed samples and 100% and $\geq 99\%$ after 24 hours at room temperature or 12 weeks at -20 °C, respectively, in unprocessed samples.

Keywords: Metoclopramide, Loratadine, Human plasma, HPLC

*Corresponding Author Email muhammad@kfshrc.edu.sa

Received 01 February 2018, Accepted 23 February 2018

Please cite this article as: Singh MN *et al.*, Development of Naringenin Nanocrystals for Enhanced Solubility and Bioavailability. American Journal of PharmTech Research 2018.

INTRODUCTION

Metoclopramide (CAS:364-62-5), 4 – Amino – 5 – chloro- N - [2 - (diethylamino) ethyl] – 2 – methoxy benzamide, is a dopamine D2 antagonist.¹ It is commonly used in treatment of nausea and vomiting associated with various conditions². It is rapidly absorbed from the gastrointestinal tract with a bioavailability of 32% to 97%³ and mean peak plasma level of 37.28 ± 9.47 ng/ml one hour after ingestion of a 10 mg therapeutic dosage⁴.

Several high-performance liquid chromatography (HPLC) methods have been reported for determination of metoclopramide level in biological samples³⁻⁷, mainly using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detector^{4,5,7}. Reported HPLC assays had a lower limit of quantification (LOQ) of 3-5 ng/ml³⁻⁶ in human plasma and 250 ng/ml in rat plasma⁷. An electron-capture gas liquid chromatography (GLC) assay reported an LOQ of 7.0 ng/ml⁸. In addition, few liquid chromatography tandem mass spectrometry (LC-MS/MS) assays have been reported⁹⁻¹¹.

In this paper, we describe a precise and accurate LC-MS/MS method for determination of metoclopramide level in human plasma with an LOQ of 0.5 ng/ml. The method is based on liquid-liquid extraction and uses 1.0 ml plasma sample. The method was fully validated and successfully used to monitor stability of metoclopramide in human plasma samples.

MATERIALS AND METHOD

Apparatus

The liquid chromatography tandem mass spectrometer (LC-MS/MS) consisted of a Water Alliance 2695 separation module equipped with Micro mass Quattro micro API bench-top triple quadrupole mass spectrometer interfaced with a Z-spray electrospray ionization (ESI) source was used. Analysis was performed on reversed phase Atlantis dC₁₈ column (2.1 x 100 mm, 3 μ m) protected by Symmetry C₁₈ guard column (3.9 x 20 mm, 5 μ m), Waters Corporation, Milford, MA, USA. Mass Lynx software (Ver 4.0) working under Microsoft Window XP professional environment was used to control the instrument parameters and for data acquisition.

Chemical and reagents

All reagents were of analytical grade unless stated otherwise. Metoclopramide hydrochloride and loratadine standards (purity ≥ 98 %) and *tert*-butyl methyl ether were obtained from Sigma-Aldrich, United Kingdom. Formic acid and acetonitrile (HPLC grade) were purchased from Fisher Scientific, NJ, USA. HPLC grade water was prepared by reverse osmosis and further purified by passing through Synergy water purification system (Millipore, Bedford, MA, USA). Drug-free human

plasma was obtained from the blood bank of King Faisal Specialist Hospital & Research Centre (KFSHRC) Riyadh, Saudi Arabia.

Chroma to graphic conditions

The mobile phase consisted of 0.4% formic acid ($\text{pH}=3.0 \pm 0.05$) and acetonitrile (20:80, v:v) and was delivered at a flow rate of 0.25 ml/minutes. The analysis was carried out at room temperature under isocratic condition. ESI was operated in the positive-ion mode at a capillary voltage of 4.0 kV and cone voltage of 30 V. Nitrogen was used as nebulizing and desolvation gas at a flow rate of 50 and 600 L/hr, respectively. Argon was used as the collision gas at a pressure of 1.28×10^{-3} mbar. The optimum collision energy for metoclopramide and the IS was 25 eV. The ion source and the desolvation temperatures were maintained at 120 °C and 350 °C, respectively. Metoclopramide and the IS were detected using positive ion multiple reaction monitoring (MRM) mode at the following transitions of mass to charge (m/z): 299.8→226.9 and 383.4→337.2, respectively.

Preparation of standard and quality control samples

Stock solutions (1 mg/ml) of metoclopramide and loratadine (internal standard, IS) were prepared in acetonitrile. They were diluted to produce working solutions of 100 ng/ml of metoclopramide in plasma and 5.0 µg/ml of IS in acetonitrile. Calibration curve standards (nine concentrations) in the range of 0.5–100 ng/ml and four quality control (QC) samples (0.5, 1.5, 50, and 90 ng/ml) were prepared in human plasma. 1.0 ml aliquots were transferred into teflon-lined, screw-capped, borosilicate glass 12 x 100 mm culture tubes (Fisher Scientific Co., Fairlawn, NJ, USA) and stored at -20 °C.

Sample preparation

Aliquots of 1.0 ml of blank plasma, calibration curves, or QC samples in culture tubes were allowed to equilibrate to room temperature (RT). To each tube, 50 µl of the IS working solution (0.25 µg) was added and the mixture was vortexed for 20 seconds. After the addition of 5 ml of *tert*-butyl methyl ether, the mixture was vortexed again for 2 minutes and then centrifuged at room temperature for 20 minutes at 4700 rpm. The clear organic supernatant layer was carefully transferred into a clean tube, dried under gentle stream of nitrogen at 40 °C, reconstituted in 80 µl acetonitrile, and transferred to an auto-sampler vial. 10 µl samples were injected into the system with a run time of 3 minutes.

Stability studies

Three QC samples (1.5, 50, and 90 µg/ml) were used for stability studies: five aliquots of each QC sample were extracted and immediately analyzed (baseline), five aliquots were allowed to stand on bench-top for 24 hours at room temperature before being processed and analyzed, five aliquots were

stored at -20 °C for 12 weeks before being processed and analyzed, and five aliquots were processed and stored at room temperature for 24 hours or at -20 °C for 48 hours before analysis. Fifteen aliquots of each QC sample were stored at -20°C for 24 hours. They were then taken out of freezer and left to completely thaw unassisted at room temperature. Five aliquots of each sample were extracted and analyzed and the rest were returned to -20 °C for another 24 hours. The cycle was repeated three times.

Method validation

The LC-MS/MS method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bio analytical method validation guidance¹². The validation parameter included specificity, linearity, accuracy, precision, recovery, and stability.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

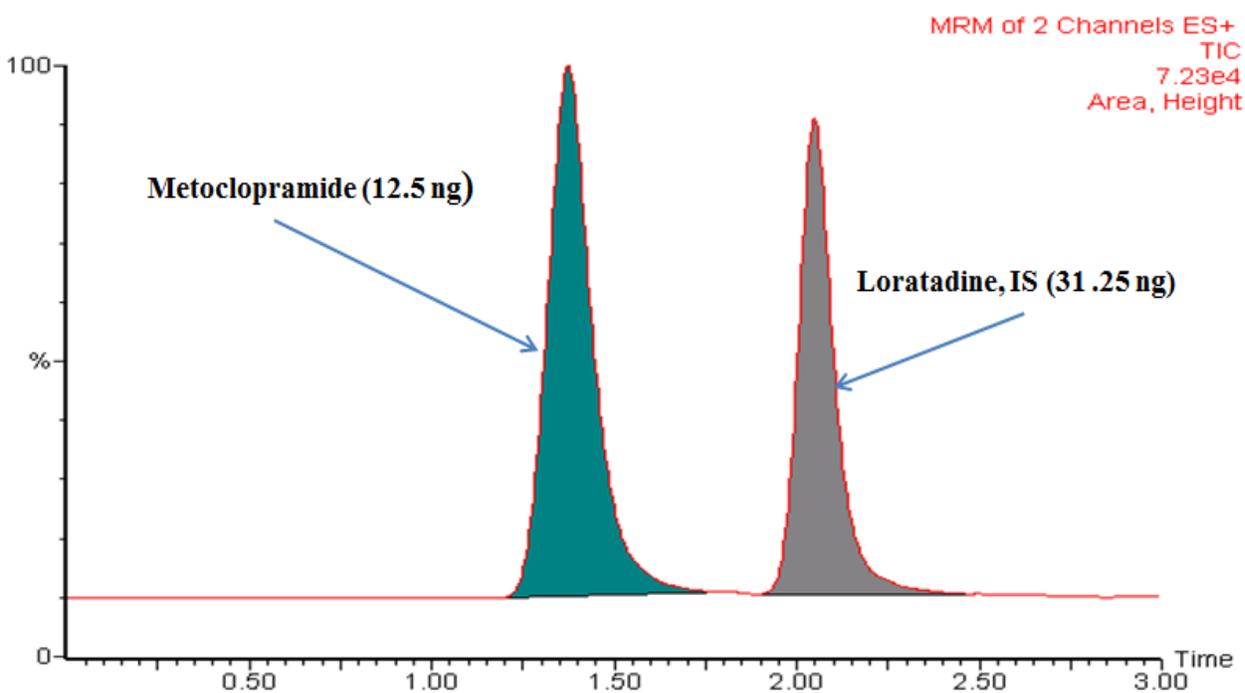


Figure 1 depicts total ion current (TIC) chromatogram of an extracted plasma sample containing 12.5 ng metoclopramide and 31.25 ng IS.

Optimal experimental conditions consisted of a mobile phase composed of 0.4% formic acid (pH=3.0 ± 0.05) and acetonitrile (20:80, v:v) and delivered at a flow rate of 0.25 ml/minute. Under these conditions metoclopramide and the IS were well separated and detected within a 3 minute run. The retention times of the metoclopramide and the IS were around 1.4 and 2.1 minute, respectively. The ESI source was operated at optimum capillary voltage of 4.0 kV and cone voltage of 30 V. The

optimum collision energy for metoclopramide and the IS was 25 eV. The ion source and the desolvation temperatures were maintained at 120 °C and 350 °C, respectively.

1.0 ml plasma samples containing 100 ng/ml metoclopramide were spiked with 50 µl of IS (5 µg/ml), extracted, and reconstituted in 80 µl of acetonitrile, and 10 µl were injected into the system.

Specificity

We screened six batches of blank human plasma and nine frequently used medications (acetaminophen, ascorbic acid, aspirin, caffeine, diclofenac, ibuprofen, nicotinic acid, omeprazole, and ranitidine) for potential interference. No interference was found in plasma, and none of the drugs co-eluted with metoclopramide or the IS.

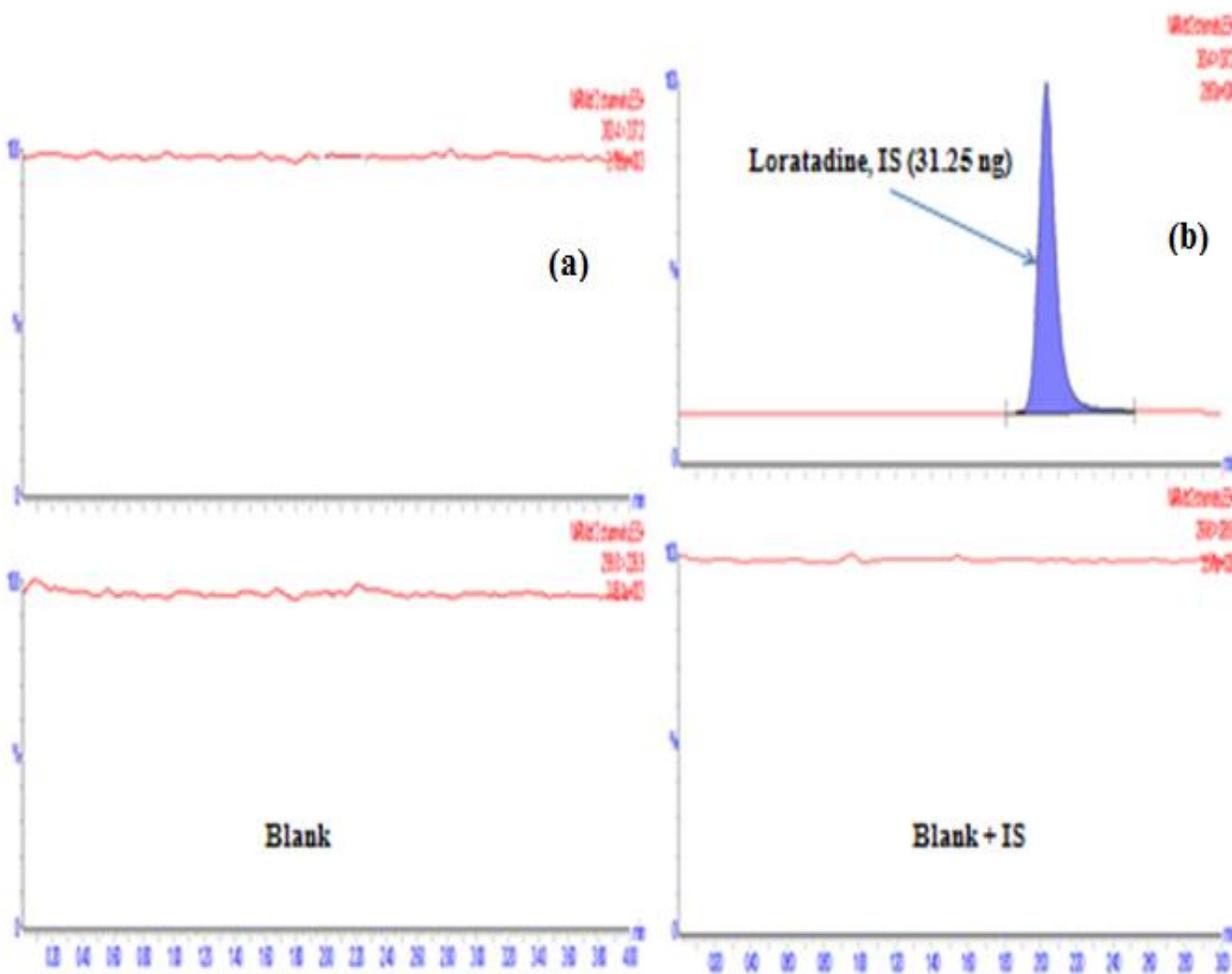


Figure 2 depicts a representative multiple reaction monitoring (MRM) chromatograms of blank and IS spiked plasma.

1.0 ml drug-free plasma samples unspiked (a) or spiked with 50 µl of IS (5 µg/ml) (b) were extracted and reconstituted in 80 µl of acetonitrile, and 10 µl were injected into the system.

Limit of detection & quantification and linearity

The limit of quantification, defined as the lowest concentration on the calibration curve that can be determined with acceptable precision and accuracy (i.e., coefficient of variation and bias $\leq 20\%$), was 0.5 ng/ml. The limit of detection (signal to noise-ratio ≥ 3) was 0.3 ng/ml.

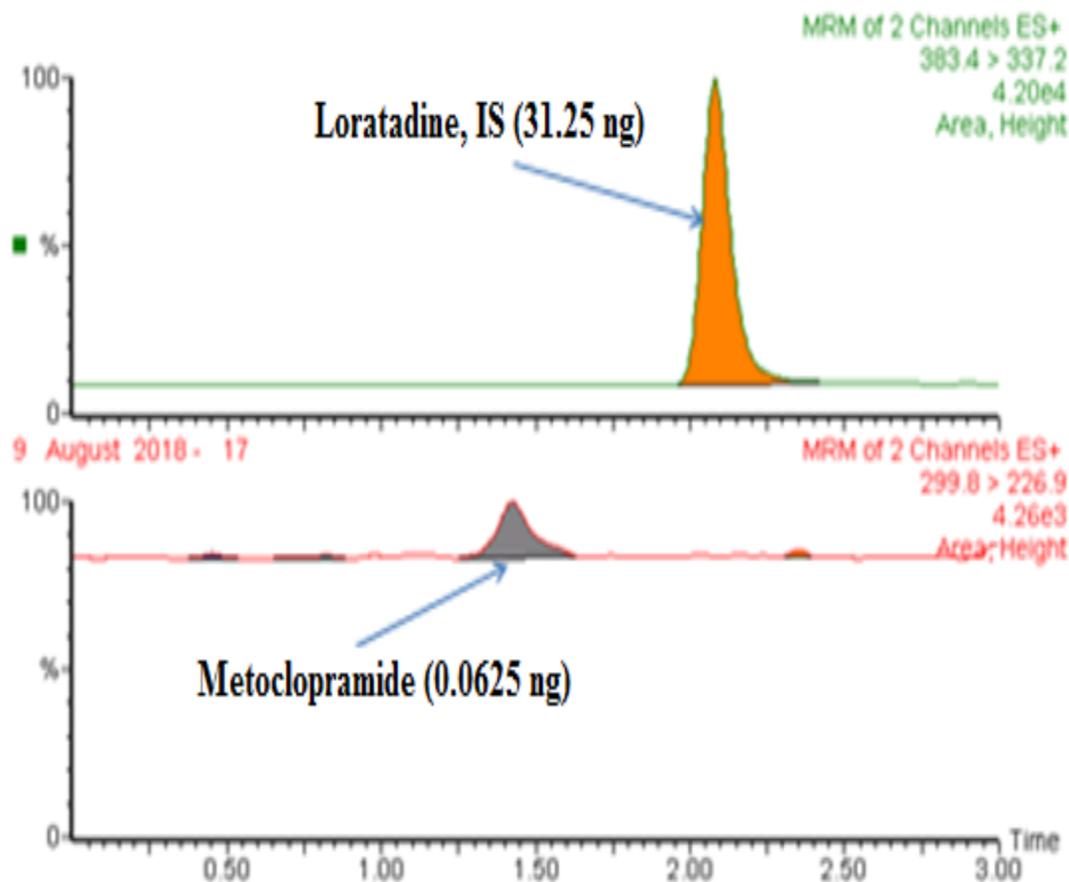


Figure 3: MRM chromatogram of an extracted plasma sample containing 0.0625 ng metoclopramide and 31.25 ng loratadine (IS).

1.0 ml plasma samples containing 0.5 ng/ml metoclopramide were spiked with 50 μ l of IS (5 μ g/ml), extracted, and reconstituted in 80 μ l of acetonitrile, and 10 μ l were injected into the system. **Figure 3** depicts an MRM chromatogram of a plasma sample containing 0.5 ng/ml metoclopramide (lowest limit of quantification). Linearity of metoclopramide was evaluated by analyzing ten curves of nine (plus zero) standard concentrations prepared in human plasma. Mean (SD) of slope, intercept, and coefficient of determination (R^2) of the ten curves were 0.0151 (0.0016), 0.0075 (0.0073), and 0.9978 (0.0009), respectively. The suitability of the calibration curves was confirmed by back-calculating the concentrations of metoclopramide (**Table 1**). All back-calculated concentrations were well within the acceptable limits (CV $\leq 8.4\%$, absolute bias $\leq 12\%$).

Table 1: Back-calculated metoclopramide concentrations from ten calibration curves

Nominal level(ng/ml)	Measured level(ng/ml)	CV (%)	Bias (%)
	Mean (SD)		
0.5	0.56 (0.05)	8.4	12.0
1.0	1.07 (0.06)	5.3	7.0
2.0	2.64 (0.16)	5.9	5.6
5.0	5.44 (0.22)	4.1	8.8
10	10.79 (0.61)	5.7	7.9
20	20.85 (1.61)	7.7	4.3
40	41.38 (3.04)	7.4	3.5
80	78.71 (3.80)	4.8	-1.6
100	100.54 (2.27)	2.3	0.5

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100.

Bias = (mean measured concentration – nominal concentration divided by nominal concentration) × 100.

Precision and bias (inaccuracy)

The intra-day and inter-day precision and bias of the method were evaluated by analyzing four QC concentrations (0.5, 1.5, 50, and 90 ng/ml). Intra-day precision and bias (n = 10) ranged from 3.2% to 14.4% and from -2.7 % to 4.0 %, respectively. Inter-day precision and bias, determined over three different days (n = 20), ranged from 5.2% to 12.0% and from 0.67 % to 6.0%, respectively. The results are summarized in **Table 2. Figure 4** depicts MRM chromatograms of three quality control samples.

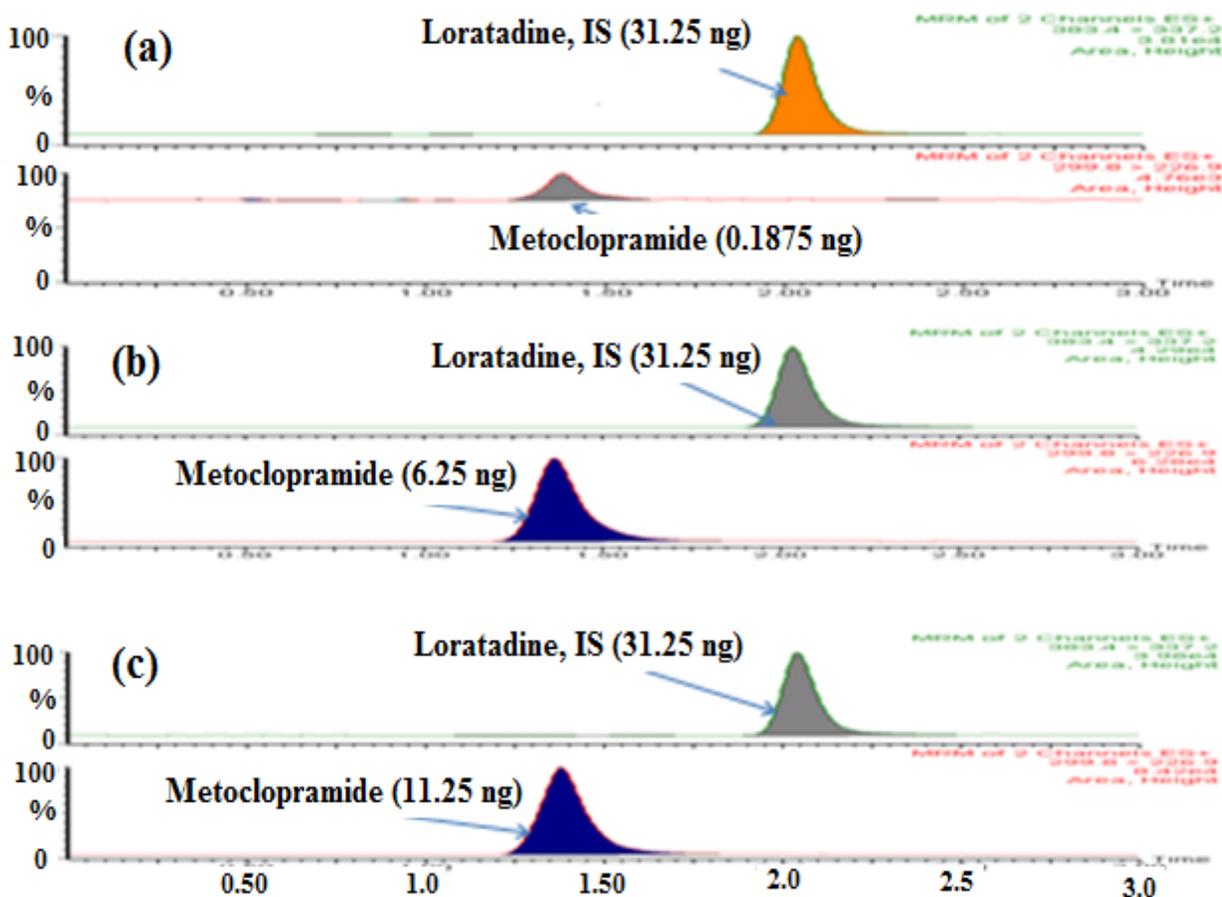


Figure 4: MRM chromatograms of three extracted quality control samples containing 0.1875, 6.25, and 11.25 ng metoclopramide and 31.25 ng loratadine (IS).

1.0 ml plasma samples containing metoclopramide 1.5 (a), 50 (b) or 90 (c) ng/ml were spiked with 50 μ l of IS (5 μ g/ml), extracted, and reconstituted in 80 μ l of acetonitrile, and 10 μ l were injected into the system.

Table 2: Intra and inter-day precision and bias of Metoclopramide assay

Nominal level (ng/ml)	Measured level (ng/ml) Mean (SD)	CV (%)	Bias (%)
Intra-day (n = 10)			
0.5	0.52 (0.08)	14.4	4.0
1.5	1.46 (0.07)	5.0	-2.7
50	49.30 (3.45)	7.0	-1.4
90	90.68 (2.94)	3.2	0.8
Inter-day (n = 20)			
0.5	0.53 (0.06)	12.0	6.0
1.5	1.49 (0.08)	5.6	0.7
50	51.55 (4.07)	7.9	3.1
90	90.72 (4.69)	5.2	0.8

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100.

Bias = (mean measured concentration – nominal concentration divided by nominal concentration) × 100.

Recovery

Extraction recovery of metoclopramide was assessed by direct comparison of peak areas from plasma and mobile phase samples, using five replicates for each of four QC samples (0.5, 1.5, 50, and 90 ng/ml). Similarly, the recovery of the IS was determined by comparing peak areas of the IS in five aliquots of human plasma spiked with 50 µl of 5.0 µg/ml IS with peak areas of equivalent concentration in acetonitrile. The results are presented in **Table 3**. Mean recovery of metoclopramide and the IS were 91% and 88%, respectively.

Table 3: Recovery of metoclopramide and the internal standard from 1.0 ml human plasma

Concentration (ng/ml)	Human plasma *Mean (SD)	Acetonitrile *Mean (SD)	Recovery (%)
Metoclopramide			
0.5	328 (32)	383 (30)	86
1.5	715 (80)	771 (50)	93
50	13672 (1312)	15081 (538)	91
90	23749 (1091)	25578 (900)	93
IS (0.25 µg/ml)	35989 (2174)	40740 (3731)	88

*Mean peak area (standard deviation), n = 5. Recovery was calculated as mean peak area in human plasma divided by mean peak area in mobile phase x 100.

Matrix effect

Matrix effect was evaluated by comparing the peak area response of pre and post extracts of metoclopramide at four concentrations (0.5, 1.5, 50, and 90 ng/ml) and the IS at 0.25 µg/ml. Twenty five extracted blank plasma samples were reconstituted using 80 µl of acetonitrile containing equal amount of metoclopramide and IS. Matrix effect, calculated as ion suppression, was -9.7% for metoclopramide and -11.7% for the IS.

Stability

Metoclopramide and IS stability in processed and unprocessed plasma samples was investigated using three QC samples (1.5, 50, and 90 ng/ml). Metoclopramide in processed samples was stable for 24 hours at room temperature (≥ 94%) or for 48 hours at -20 °C (≥ 95%). Metoclopramide in unprocessed plasma samples was stable for 24 hours at room temperature (100%), 12 weeks at -20 °C (≥ 99%), or after three freeze-and thaw cycles (≥ 97%). **Table 4** summarizes the results of stability studies.

Table 4: Stability for metoclopramide in human plasma

Stability Nominal level (ng/ml)	Stability (%)						
	Unprocessed		Processed		Freeze-Thaw Cycle		
	24 hrs RT	12 wks	24 hrs-20 °C	RT-20 °C	1	2	3
1.5	110	102	97	98	101	111	102
50	106	100	108	109	106	114	109
90	101	99	94	95	97	100	98

Stability (%) = mean measured concentration (n = 5) at the indicated time divided by mean measured concentration (n = 5) at baseline x 100. Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), or after freezing at -20 °C for 12 weeks (12 wks, -20 °C), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs, RT) or 48 hours at -20 °C (48 hrs, -20 °C). Freeze-thaw (FT), samples were frozen at -20 °C and thaw at RT.

CONCLUSION

The described LC-MS/MS assay for the determination of metoclopramide level in human plasma was found to be simple, sensitive, specific, precise, and accurate. It requires 1.0 ml plasma and 3 minutes run time. It was successfully applied to monitor stability of metoclopramide under various conditions commonly encountered in the clinical laboratories.

REFERENCES

1. Metoclopramide hydrochloride". Monograph. The American Society of Health-System Pharmacists. Retrieved 2014; 9-27.
2. Rao AS, Camilleri M "Review article: metoclopramide and tardive dyskinesia". *Aliment. Pharmacol Ther* 2010; 31 (1): 11–19.
3. Javanbakht M, Shaabani N, Akbari Adergani B. Novel molecularly imprinted poly "mers for the selective extraction and determination of metoclopramide in human serum and urine samples using high-performance liquid chromatography. *J Chrom B* 2009; 877 (24): 2537-2544.
4. Buss DC, Hutchings AD, Scott S, Rout ledge PA. A rapid liquid chromatographic method for the determination of metoclopramide in human plasma. *Thera Drug Mont* 1990; 12(3):293-296.
5. El-Sayed YM, Khidr SH, Niazy EM. A rapid and sensitive high-performance liquid chromatographic method for the determination of metoclopramide in plasma and its use in pharmacokinetic studies. *J Anal Letter* 1994; 27 (1): 55-70.

6. Henryk Lamp arczyk H, Chmielewska A, Konieczna L, Plenis A, Zarzycki PK. RP-HPLC method with electrochemical detection for the determination of metoclopramide in serum and its use in pharmacokinetic studies. *Biomed Chrom* 2002; 15 (8): 513-517.
7. Radwan MA. Determination of metoclopramide in serum by HPLC assay and its application to pharmacokinetic study in rat. 1998; 31 (14): 2397-2410.
8. Tam YK, Axelson JE, Ongley R. Modification of metoclopramide GLC assay: Application to human biological specimens. *J Pharm Sci.* 1979; 68(10):1254-6.
9. Lee HW, Ji HY, Kim HY, Park ES, Lee KC, Lee HS. Determination of metoclopramide in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry. *J Chrom B* 2009; 877 (18-19): 1716-1720.
10. Yan M, Li HD, Chen BM, Liu XL, Zhu YG. Determination of metoclopramide in human plasma by LC-ESI-MS and its application to bio equivalence studies. *J Chrom B Ana Tech Biomed Life Sci* 2010; 1; 878 (11-12):883-887.
11. Khan A, Khan J, Irfan M, Naqvi SBS, Khan GM, Shoaib MH, Yousaf RI, Khan A. Validation and application of high performance liquid chromatographic method for the estimation of metoclopramide hydrochloride in plasma. *Pak J Pharm Sci.* 2017; 30(1):143-147.
12. Bioanalytical Method Validation, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), June 2018.

AJPTR is

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: editor@ajptr.com

