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Determination of metoclopramide in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry

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ABSTRACT

For the rapid, selective and sensitive analysis of metoclopramide in human plasma, hydrophilic interaction chromatography with electrospray ionization tandem mass spectrometric (HILIC/MS/MS) method was developed. This method involved liquid–liquid extraction with dichloromethane followed by separation on an Atlantis HILIC silica column using the mobile phase of acetonitrile–ammonium formate (100 mM, pH 6.5) (85:15, v/v). Analytes were quantified using electrospray ionization mass spectrometry in the selected reaction monitoring mode. The standard curve was linear (r^2 = 0.998) over the concentration range of 2.00–150 ng/mL using 50 μL of plasma sample. The coefficient of variation and relative error for intra- and inter-assay at four QC levels were 1.8–7.7% and −7.5 to 3.6%, respectively. The matrix effect for metoclopramide and levosulpiride (internal standard) was practically absent. The present method was successfully applied to the pharmacokinetic study of metoclopramide after oral dose of metoclopramide hydrochloride (10 mg) to male healthy volunteers.

1. Introduction

Metoclopramide, 4-amino-5-chloro-*N*-(2-diethylamino-ethyl)-2-methoxy-benzamide, is primarily used in adults and children as an antiemetic drug or a gastrointestinal prokinetic drug [1]. Metoclopramide is extensively metabolized to deethyl-metoclopramide by CYP2D6 and also conjugated to metoclopramide *N*-4-sulfate and *N*-glucuronide [2].

For the quantification of metoclopramide in biological fluids, gas chromatography/mass spectrometry (GC/MS) [3–5] and high performance liquid chromatography (HPLC) methods with UV [6–14], fluorescence [15,16] or electrochemical detection [17] have been reported. Normal-phase HPLC using a silica column [6,7] and reversed-phase (RP) HPLC [9,10,12–16] or ion-pair HPLC methods on octadecyl column [8,11,17] were described for the analysis of metoclopramide in biological fluids. To the best of our knowledge, the LC/MS method for the determination of metoclopramide in biological fluids has not been described. The sample preparation procedures for the extraction of metoclopramide from biological matrix consisted of protein precipitation [16], solid-phase extraction [9], liquid–liquid extraction (LLE) [3–8,10,11,13–15], on-line trace enrichment [12] or combination of protein precipitation with SPE [17]. Those methods use a large amount of biological samples (0.5–2.0 mL plasma or urine samples) in order to obtain the high sensitivity or include relatively time-consuming extraction procedures and long analysis time.

Hydrophilic interaction chromatography (HILIC) technique using bare silica or polar bonded phase and low aqueous/high organic mobile phase has been shown to be a valuable tool for the quantitative analysis of the polar compounds in biological samples [18–24]. In view of LC/MS analysis, the higher organic content in the mobile phase of HILIC resulted in the sensitivity improvement and less matrix effect compared to RP-HPLC [18–24].

In this study, the use of HILIC/MS/MS on a silica column with high organic/low aqueous mobile phase is described to determine the polar metoclopramide in human plasma. The rapid, robust and sensitive HILIC/MS/MS method using LLE with dichloromethane as sample preparation procedure was validated for the quantification of metoclopramide using 50 μL of human plasma. The present method has been successfully applied to the pharmacokinetic study of metoclopramide after oral administration of a metoclopramide tablet in humans.

2. Experimental

2.1. Materials and reagents

Metoclopramide (purity, >99%) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Levosulpiride (purity, >99%; inter-

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nal standard) was a gift from Dong-A Pharm. Co. (Yongin, Korea). Acetonitrile and dichloromethane (HPLC grade) were obtained from Burdick & Jackson Inc. (Muskegon, MI, USA) and the other chemicals were of the highest quality available. Drug-free human plasma containing sodium heparin as the anticoagulant was obtained from healthy volunteers.

2.2. Preparation of calibration standards and quality control samples

Primary stock solutions of metoclopramide and levosulpiride (1 mg/mL) were prepared in dimethylsulfoxide. Working standard solutions of metoclopramide were prepared by diluting primary stock solution with acetonitrile. The levosulpiride working solution (internal standard, 1 μg/mL) was prepared by diluting an aliquot of primary stock solution with acetonitrile. All standard solutions were stored at ca. 4 °C in 20 mL scintillation vial in the dark when not in use.

Human plasma calibration standards of metoclopramide (2.00, 4.00, 10.0, 50.0, 100, 120 and 150 ng/mL) were prepared by spiking the working standard solutions into a pool of drug-free human plasma. Quality control (QC) samples at 2.00, 3.00, 40.0 and 140 ng/mL were prepared in bulk by adding 125 μL of the appropriate working standard solutions (40, 60, 800 and 2800 ng/mL) to drug-free human plasma (2375 μL). The bulk samples were aliquoted (50 μL) into polypropylene tubes and stored at −80 °C until analysis.

2.3. Sample preparation

Fifty microliters of human blank plasma, calibration standards and QC samples were vortex-mixed with 5 μL of levosulpiride working solution and 1000 μL of dichloromethane in 1.5-mL polypropylene tubes. The mixtures were centrifuged at 10,000 × *g* for 5 min. 900 μL of the organic layer was pipette-transferred and evaporated to dryness using a vacuum concentrator (EZ-plus, Genevac, UK) for 20 min at 30 °C. The residues were dissolved in 400 μL of 85% acetonitrile in water by sonicating for 3 min and centrifuged. The aliquots (2 μL) were injected onto LC/MS/MS system.

2.4. HILIC/MS/MS analysis

The LC/MS/MS system consisted of a Nanospace SI-2 pump, SI-2 column oven, an SI-2 autosampler (Shiseido, Tokyo, Japan) and a tandem quadrupole mass spectrometer (TSQ Quantum Access, ThermoFisher Scientific, CA, USA). The separation was performed on a Atlantis HILIC silica column (3 μm, 3.0 mm i.d. × 50 mm, Waters, CA, USA) using a mixture of acetonitrile–ammonium formate (100 mM, pH 6.5) (85:15, v/v) at a flow rate of 0.5 mL/min. The column and autosampler tray temperatures were 50 and 6 °C, respectively. The analytical run time was 3.5 min. For the quantification of metoclopramide and levosulpiride, selected reaction monitoring (SRM) mode was employed using the electrospray ionization in positive mode. The parameters of the mass spectrometer are summarized in Table 1. The LC/MS/MS system was controlled by the Xcalibur® software (ThermoFisher Scientific) and data were collected with the same software.

2.5. Method validation

Batches, consisting of triplicate calibration standards at each concentration, were analyzed on three different days to complete the method validation. In each batch, QC samples at 2.00, 3.00, 40.0 and 140 ng/mL were evaluated in sets of six replicates to evaluate the intra- and inter-day precision and accuracy. The percentage deviation of the mean from true values, expressed as relative error (RE), and the coefficient of variation (CV) serve as the measure of accuracy and precision, respectively.

Table 1
Mass spectrometer parameters for the analysis of metoclopramide and levosulpiride.

Parameter	Value
Spray voltage (V)	5000
Vaporizer temperature (°C)	250
Capillary temperature (°C)	330
Sheath gas pressure (psi)	35
Auxiliary gas pressure (psi)	10
Tube lens offsets (V)	83 for metoclopramide 96 for levosulpiride
Collision energy (V)	18 for metoclopramide 30 for levosulpiride
Dwell time (s)	0.5
Ion transition for	
Metoclopramide (*m*/*z*)	300.13 → 226.79
Levosulpiride (*m*/*z*)	342.04 → 112.32

The absolute and relative matrix effects for metoclopramide and levosulpiride were assessed by analyzing the two sets of the standards at four concentrations, i.e., 2.00, 3.00, 40.0 and 140 ng/mL according to the approach of Matuszewski et al. [25]. The absolute matrix effect for metoclopramide and levosulpiride was assessed by comparing the mean peak areas of the analyte spiked at four concentrations into the extracts originated from five different lots of blank human plasma samples (set 2) to the mean peak areas for neat solutions of the analytes in 85% acetonitrile in water (set 1). The variability in the peak areas of the analyte spiked post-extraction into five different plasma extracts (set 2) expressed as CVs (%), was considered as a measure of the relative matrix effect.

Recovery of metoclopramide was evaluated by comparing mean peak areas of metoclopramide spiked before liquid–liquid extraction into the same five different sources as set 2 (set 3) to those of metoclopramide spiked post-extraction into different blank plasma lots at four concentrations (set 2).

To evaluate the three freeze/thaw cycle stability and room temperature storage stability, six replicates of QC samples at each of the low and high concentrations (3.00 and 140 ng/mL, respectively) were subjected to three freeze/thaw cycles or storage at room temperature for 4 h before processing. Six replicates of QC samples at each of the low and high concentrations were processed and stored under autosampler conditions for 24 h and were assayed to evaluate post-preparative stability.

2.6. Clinical application

The present method was applied to a pharmacokinetic study after an oral administration of metoclopramide hydrochloride to male volunteers. The protocol was approved by an institutional review board at the Research Institute for Drug Development, Sungkyunkwan University (Suwon, Korea) and the informed consent was obtained from the subjects after explaining the nature and purpose details of the study. Four healthy volunteers, fasted for 24 h, received a single oral dose of metoclopramide hydrochloride (10 mg tablet) with 200 mL of water. Blood samples (2 mL) were withdrawn from the forearm vein at 0 (control), 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8 and 12 h post-dosing, transferred to Vacutainer™ plasma glass tubes (sodium heparin, BD, NJ, USA), and centrifuged at 3000 × *g* for 10 min. The plasma samples were transferred to polypropylene tubes and stored at −80 °C until analysis.

The maximum plasma concentration (C_{max}) and the time to maximum concentration (T_{max}) were determined by visual inspection from each volunteer's plasma concentration–time curve for metoclopramide. Area under the plasma concentration–time curve (AUC) from time zero to time infinity and terminal elimination

half-life ($t_{1/2}$) were calculated using a non-compartment analysis (WinNonlin, Pharsight, Mountain View, CA, USA).

3. Results and discussion

3.1. HILIC/MS/MS

The electrospray ionization of metoclopramide and levosulpiride produced the abundant MH^+ ions at m/z 300.13 and 342.04, respectively, without the adduct formation and fragmentation. MH^+ ions of metoclopramide and levosulpiride were selected as the precursor ions and subsequently fragmented in MS/MS mode to obtain the product ion spectra, yielding the useful structural information (Fig. 1). The prominent product ion for metoclopramide was m/z 226.79 due to the loss of 2-diethylamino group from MH^+ ion and levosulpiride produced the major product ion at m/z 112.32 via the loss of 2-methoxy-5-sulfamoyl-benzamide from MH^+ ion. Quantification of the analytes was performed using the SRM mode due to the high selectivity and sensitivity of SRM data acquisition, where the transition of the precursor ion to a product ion is monitored: m/z 300.13 → 226.79 for metoclopramide and m/z 342.04 → 112.32 for levosulpiride (internal standard).

The selectivity of HILIC separation can be drastically changed by the judicious selection of column and the nature of buffer and mobile phase pH [18–24]. The use of pH 6.5 ammonium formate buffer resulted in the best retention and sensitivity of metoclopramide and levosulpiride compared to the use of ammonium formate at pH 3.0 and 4.5 on an Atlantis HILIC silica column. Metoclopramide showed the better retention and peak shape on an Atlantis HILIC silica column compared to a Luna HILIC silica column. The high organic content in the mobile phase of HILIC resulted in the high sensitivity of metoclopramide in MS/MS detection due to the high efficiency of spraying and desolvation techniques. The plasma sample volume (50 μL) used in this study was smaller than those (500–2000 μL) in other GC/MS [3–5] and RP-HPLC methods [6–17] to obtain the limit of quantification (LOQ) of 2.00 ng/mL.

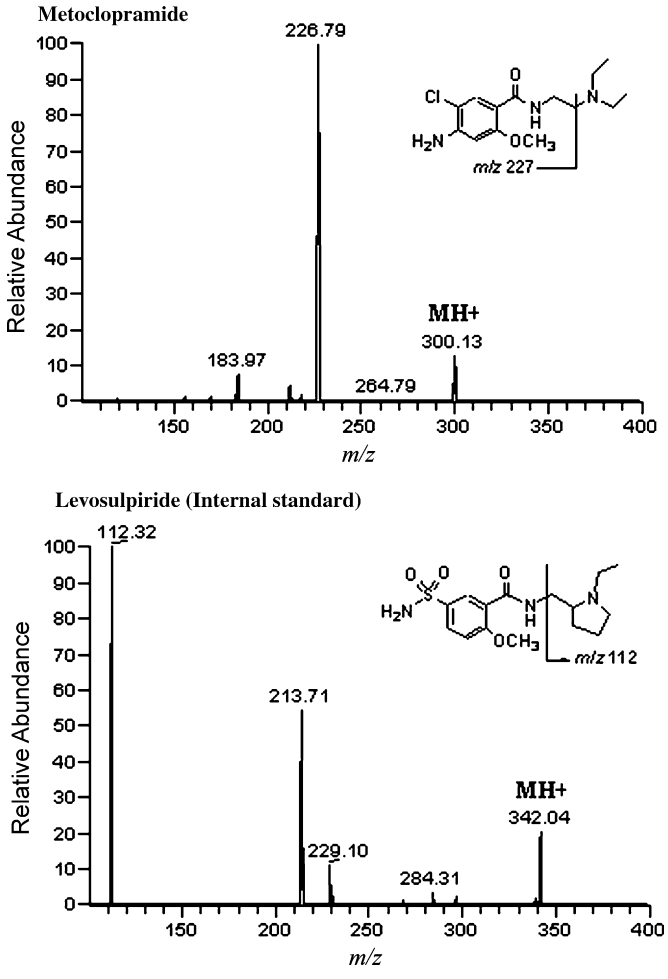

Fig. 1. Product ion mass spectra of (a) metoclopramide and (b) levosulpiride.

There was no interference peak at the retention times of metoclopramide (1.9 min) and levosulpiride (2.5 min) in the analysis of blank plasma samples obtained from 10 humans, confirming the specificity of the present method (Fig. 2). The retention times of metoclopramide and levosulpiride were reproducible throughout the experiment and no column deterioration was observed after analysis of 400 human plasma samples. Sample carryover effect was not observed.

3.2. Linearity

Calibration curves were obtained over the concentration range of 2.00–150 ng/mL for metoclopramide in human plasma. Linear regression analysis with a weighting of 1/concentration gave the optimum accuracy (RE, −2.3 to 1.5%) and precision (CV, 4.0–9.5%) of the corresponding calculated concentrations at each level (Table 2). The low CV value (3.4%) for the slope indicated the repeatability of the method (Table 2).

3.3. Precision and accuracy

Table 3 shows a summary of intra- and inter-day precision and accuracy data for QC samples containing metoclopramide. Both intra-and inter-day CV values ranged from 1.8 to 7.7% at four QC levels. The intra- and inter-assay RE values were −7.5 to 3.6% at four QC levels. These results indicated that the present method has the acceptable accuracy and precision. The lower limit of quantification (LOQ) for metoclopramide was set at 2.00 ng/mL using 50 μL of human plasma. Representative chromatogram at the LOQ is shown in Fig. 2b and the signal-to-noise ratio for metoclopramide are higher than 20.

3.4. Matrix effect and recovery

The mean absolute matrix effect, the peak areas of set 2 to those of set 1 multiplied by 100, at four concentrations was 99.4 and 100.2% for metoclopramide and levosulpiride, respectively (Table 4). A value of 100% indicates that the response in the solvent and in the plasma extracts were the same and no absolute matrix effect was observed. A value of <100% indicates an ionization suppression and a value of >100% indicates an ionization enhancement. There was little absolute matrix effect for metoclopramide and levosulpiride. The relative matrix effect was assessed as CVs in the peak areas of metoclopramide and levosulpiride spiked into each plasma extract originated from five human sources (set 2). The CVs for metoclopramide at four concentrations in set 2 were 1.2–4.9% and seemed to be comparable to CVs of peak areas of standards injected directly in 85% acetonitrile in water (set 1) (1.4–2.7%), indicating that there was no relative matrix effect for metoclopramide.

The overall extraction recovery of metoclopramide from human plasma was 76.5% at four concentration levels and the recovery of levosulpiride (internal standard) was 78.0% (Table 4). The liquid–liquid extraction using dichloromethane at neutral pH has been successfully applied to the extraction of metoclopramide from human plasma.

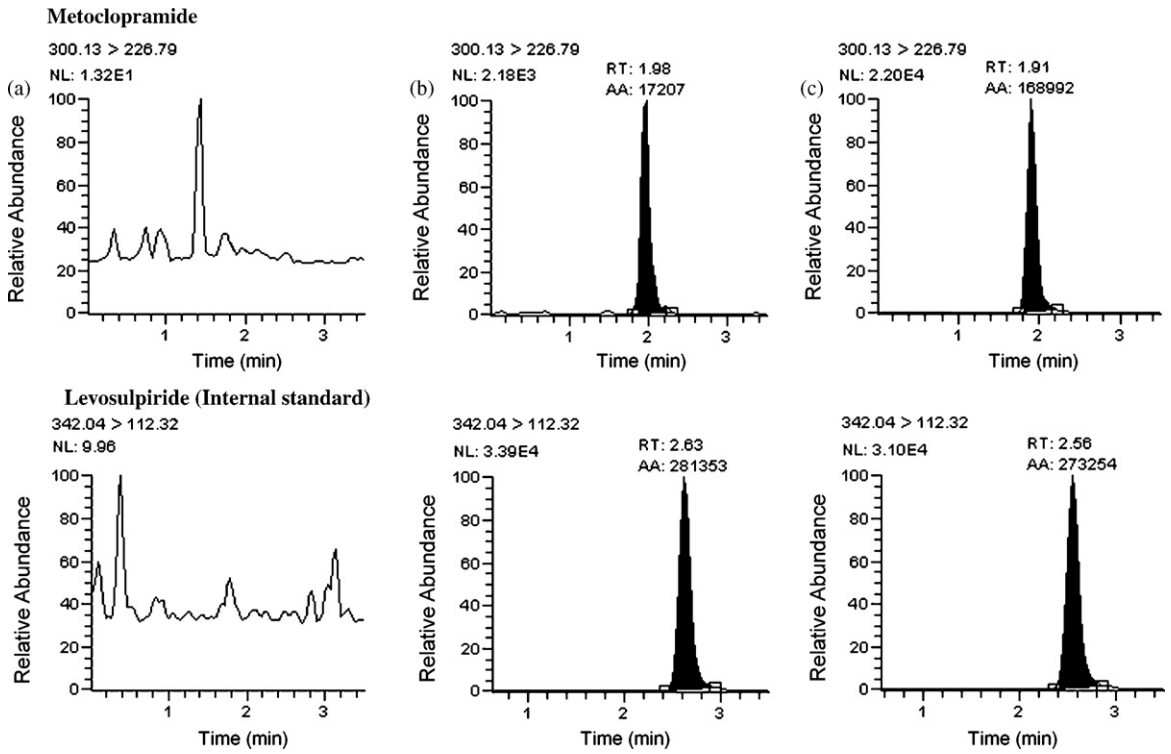

Fig. 2. SRM chromatograms of (a) a human blank plasma, (b) a human plasma sample spiked with 2.00 ng/mL of metoclopramide and (c) a human plasma sample obtained 2 h after oral administration of metoclopramide hydrochloride (10 mg) to a male volunteer.

Table 2
Calculated concentrations of metoclopramide in calibration standards prepared in human plasma ($n = 3$).

Statistical variable	Theoretical concentration (ng/mL)							Slope	Intercept	r^2
	2.00	4.00	10.0	50.0	100	120	150			
Mean (ng/mL)	2.03	4.00	9.88	50.3	97.7	120	152	0.0284	0.0053	0.9980
CV (%)	9.5	5.4	4.5	4.5	4.4	4.0	4.0	3.4		
RE (%)	1.5	0.0	−1.2	0.6	−2.3	0.0	1.3			

Table 3
Precision and accuracy of metoclopramide in quality control samples.

Statistical variable	Intra-day ($n = 6$)				Inter-day ($n = 3$)			
QC (ng/mL)	2.00	3.00	40.0	140	2.00	3.00	40.0	140
Mean (ng/mL)	1.88	2.93	41.3	145	1.85	2.91	40.4	138
CV (%)	7.3	7.7	1.8	4.9	7.6	6.2	6.1	7.4
RE (%)	−6.0	−2.3	3.2	3.6	−7.5	−3.0	1.0	−1.4

3.5. Stability

The stabilities of processing (three freeze–thaw cycles and short-term storage at room temperature) and chromatography (reinjection) were tested and shown to be of insignificant effect (Table 5). Three freeze–thaw cycles and a 4-h storage at room temperature of QC samples at the low and high concentrations before analysis had little effect on the quantification. Extracted QCs and

Table 4
Absolute matrix effect and recovery of metoclopramide and levosulpiride (I.S.) in five different lots of human plasma.

Nominal concentration (ng/mL)	Absolute matrix effect[a] (%)		Recovery[b] (%)	
	Metoclopramide	Levosulpiride	Metoclopramide	Levosulpiride
2.00	98.4	100.7	75.3	75.1
3.00	97.3	99.6	77.4	76.9
40.0	99.5	97.6	78.3	81.9
140	102.5	102.9	75.1	78.2
Mean	99.4	100.2	76.5	78.0

[a] Absolute matrix effect expressed as the ratio of the mean peak area of an analyte spiked post-liquid–liquid extraction (set 2) to the mean peak area of same analyte standards (set 1) multiplied by 100.

[b] Recovery calculated as the ratio of the mean peak area of an analyte spiked before liquid–liquid extraction (set 3) to the mean peak area of an analyte spiked after liquid–liquid extraction (set 2) multiplied by 100.

Table 5
Stability of samples ($n = 6$).

Statistical variable	Theoretical concentration (ng/mL)	
	3.00	140
Three freeze/thaw cycles		
Mean (ng/mL)	2.84	127
CV (%)	4.8	4.4
RE (%)	−5.3	−9.3
4-h storage at room temperature		
Mean (ng/mL)	2.87	132
CV (%)	9.0	1.6
RE (%)	−4.3	−5.7
Post-preparative stability (24 h at 4 °C)		
Mean (ng/mL)	3.09	143
CV (%)	4.7	3.4
RE (%)	3.0	2.1

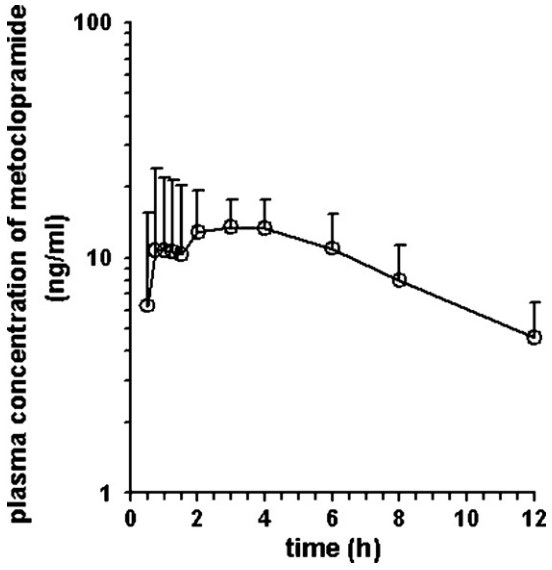

Fig. 3. Mean plasma concentration–time plot of metoclopramide after a single oral dose of metoclopramide hydrochloride (10 mg) to four male volunteers. Each point represents the mean ± S.D.

calibration standards were allowed to stand at 4 °C for 24 h prior to injection without affecting the quantification.

3.6. Clinical application

The present method has been successfully applied to the pharmacokinetic study of metoclopramide in four healthy male volunteers. Representative chromatograms of the extract of a plasma sample obtained 2 h after an oral dosing of metoclopramide hydrochloride to human are shown in Fig. 2c. After oral administration of metoclopramide hydrochloride (10 mg, Macperan®, Donghwa Pharm. Co., Anyang, Korea), plasma concentrations of metoclopramide declined in a monoexponential fashion (Fig. 3) with the mean pharmacokinetic parameters of C_{max}, 18.1 ± 6.5 ng/mL; T_{max}, 2.4 ± 2.4 h; AUC, 144.6 ± 60.0 ng h/mL and $t_{1/2}$, 4.9 ± 0.8 h. According to the report of Vlase et al. [16], C_{max}, T_{max}, AUC and $t_{1/2}$ obtained after oral administration of 20 mg metoclopromide in male Romanian volunteers were 44.0 ± 14.5 ng/mL, 1.15 ± 0.46 h, 312.6 ± 112.7 ng h/mL and 5.52 ± 1.12 h, respectively and were comparable to those obtained in this study.

4. Conclusion

A rapid, sensitive, selective and reliable HILIC/MS/MS method for the determination of metoclopramide in human plasma has been successfully developed and validated using LLE as a sample preparation procedure. This assay method demonstrated the acceptable sensitivity (LOQ: 2.00 ng/mL), precision, accuracy, selectivity, recovery and stability, and less absolute and relative matrix effects. This method was successfully applied to the determination of metoclopramide in human plasma samples obtained after an oral administration of 10 mg metoclopramide hydrochloride.

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