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# Determination of metoclopramide in human plasma by LC–ESI-MS and its application to bioequivalance studies

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ABSTRACT

An LC–MS method for the determination of metoclopramide in human plasma was developed and validated. Sample preparation involved extraction with ethyl acetate. Chromatographic separation was performed on a Thermo Hypersil-Hypurity $C_{18}$ (150 mm × 2.1 mm, 5 μm) with the mobile phase consisting of 40 mM ammonium acetate–methanol–acetonitrile. A single-quadrupole mass spectrometer with an electrospray interface was operated in the selected-ion monitoring mode to detect the $[M+H]^+$ ions at *m/z* 300 for metoclopramide and at *m/z* 384 for the internal standard (prazosin). The method was validated over 0.78–50.00 ng $mL^{-1}$ for metoclopramide. The recovery was 67.8–83.1%, and the limit of quantitation (LOQ) detection was 0.78 ng $mL^{-1}$ for metoclopramide. The intra- and inter-day precision of the method at three concentrations was 5.0–13.6% with accuracy of 99.2–104.0%. Stability of compounds was established in a battery of stability studies. The method was successfully applied to bioequivalence studies of metoclopramide hydrochloride tablets to obtain the pharmacokinetic parameters.



## 1. Introduction

Metoclopramide, a dopamine-receptor antagonist active on gastrointestinal motility, is used as an anti-emetic in the treatment of some forms of nausea and vomiting and to increase gastrointestinal motility. It is also used at much higher doses for the prevention of cancer chemotherapy-induced emesis [1].

Early publications have described methods of analyzing metoclopramide in biological samples. The techniques adopted include gas chromatography–mass spectrometry (GC–MS) [2–4], electrogenerated chemiluminescence (ECL) [5] and high performance liquid chromatography (HPLC) methods with UV [6–14], fluorescence [15] or electrochemical detection [16]. The above methods have poor sensitivity and specificity for the determination of metoclopramide in plasma after administration of low dosages. Recently, Cossu et al. [17] developed a more sensitive HPLC method to determine metoclopramide in canine plasma. Although, the obtained LOQ (0.2 ng $mL^{-1}$) is lower than the LOQ presented in this work, the metoclopramide mean retention time (RT) was too long (8.72 min). A SPE-HPLC method based on molecularly imprinted polymers (MIPs) for the extraction of metoclopramide from aqueous solutions has been developed by Javanbakht et al. [18], the limits of detection of metoclopramide in human serum and urine were 3 and 1.2 ng $mL^{-1}$, respectively. This method allowed cleaner extracts to be obtained and interfering peaks arising from the complicated biologic samples to be suppressed. But the method is time-consuming (RT = 4.65 min), and the molecular imprinting technology is not well developed in many clinical laboratories.

During the last few years, mass spectrometry has repeatedly been proven to be a powerful technique for the rapid, quantitative determination of drugs and metabolites in physiologic fluids. To the best of our knowledge, the LC–MS method for the determination of metoclopramide in biological fluids has not been described. Only an assay using hydrophilic interaction chromatography–tandem mass spectrometry (HILIC–MS–MS) was reported [19], and the LOQ of the method was 2.00 ng $mL^{-1}$. However, this method requires expensive instruments and could not be used easily in most laboratories to analyze the samples in batches. Our study focused on LC–MS because of its wider availability in ordinary laboratories as well as its sufficient sensitivity, selectivity and effectivity for the present bioequivalence studies of metoclopramide.

## 2. Experimental

### 2.1. Chemicals and reagents

Metoclopramide hydrochloride standard (purity >99.7%) was kindly supplied by Vickmans Lab. Ltd., HK. Prazosin hydrochloride

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(internal standard, IS, purity >99.7%) was purchased from Daxinan Pharmaceutical Ltd., Chengdu, China. Acetonitrile was supplied by Kermel Chemical Reagents Development Center (Tianjin, China). HPLC grade methanol was obtained from Tedia Co. (Fairfield, OH, USA). Ultra-pure water prepared by a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA) was used as the mobile phase of LC–MS. Acetonitrile and methanol were LC grade, all other chemicals and solvents were of the highest analytical grade available. Reference formulations (Metocloramide Tab®, Alpharma Inc., UK) and test formulations (Metomid Tab®, Vickmans Lab. Ltd., Hong Kong) containing 10 mg metoclopramide per tablet were used in this study.

### 2.2. Equipment

The LC system consisted of an LC-10AD *VP* pump, an SCL-10AD *VP* system controller, a CTO-10A *VP* column temperature oven, an FCV-10AD *VP* low pressure gradient unit and a DGU-14A degasser (Shimadzu, Kyoto, Japan). The single-quadrupole mass spectrometer was a Shimadzu LCMS-2010 equipped with an electrospray ionization interface. The data was collected and processed using LCMSsolution software.

### 2.3. Chromatographic conditions

Chromatographic separation was achieved on a Thermo Hypersil-Hypurity C18 column (150 mm × 2.1 mm, i.d., 5 μm, USA). The oven temperature was set at 45 °C. The mobile phase was 40 mM ammonium acetate (pH 3.5): methanol:acetonitrile = 75:5:20 at a flow rate of 0.24 mL $min^{-1}$.

### 2.4. Mass spectrometer conditions

An LCMS-2010 quadrupole mass spectrometer was interfaced with an electrospray ionization (ESI) probe. The temperatures were maintained at 250, 250 and 200 °C for the probe, CDL and block, respectively. The voltages were set at 4.5 kV, −35 V, 25 V, 150 V, and 1.7 kV for the probe, CDL, Q-array 1, 2, 3 bias, Q-array RF and detector. The flow rates of nebulizer gas and dried gas were set at 1.5 and 10 L $min^{-1}$. The ions chosen for selective monitoring were decided by positive scanning from *m*/*z* 100 to 800. For the quantification of metoclopramide, the protonated molecule ions of metoclopramide at *m*/*z* 300 $[M+H]^+$ and prazosin (IS) at *m*/*z* 384 $[M+H]^+$ were monitored. Tuning of the mass spectrometer was performed with the autotuning function of the LCMSsolution software (Version 2.04) using tuning standard solution (polypropylene glycol).

### 2.5. Analytical procedure

#### 2.5.1. Preparation of stock solutions, calibration standard and quality control samples

All concentrations of the two standards refer to the free bases. Primary stock solutions of metoclopramide (127 μg $mL^{-1}$) were prepared in methanol. Internal standard (prazosin) was also prepared as a stock solution (110 μg $mL^{-1}$) in methanol and was further diluted with methanol to give a concentration of 110 ng $mL^{-1}$ and used for all analyses. All stock solutions were stored at 4 °C before use. Calibration standards of metoclopramide were prepared by spiking the appropriate amount of the stock solution into the blank plasma obtained from volunteers (heparin was also used as anti-coagulent), at 0.78, 1.56, 3.13, 6.25, 12.50, 25.00, and 50.00 ng $mL^{-1}$, respectively, and it was mixed well. Prepared calibration curves covered the range 0.78–50.00 ng $mL^{-1}$. The quality control (QC) samples were prepared by the same way at the final concentrations were 1.56, 6.25 and 25.00 ng $mL^{-1}$. All plasma samples were stored at −20 °C, and the standard stock solutions were prepared once a month and stored at −20 °C.

#### 2.5.2. Sample preparation and extraction procedures

Frozen human plasma samples were thawed at ambient temperature. A plasma sample (0.4 mL) was placed in a 2 mL Eppendorf tube. A 50 μl aliquots of prazosin (I.S. 50.00 ng $mL^{-1}$) standard solution was added to each plasma sample and vortex-mixed. The plasma was then made alkaline by adding 100 μl NaOH solution (1 M). After a thorough vortex mixing for 30 s, the mixture was extracted with 1 mL ethyl acetate, vortex-mixed for 3 min, and centrifuged at 14,000 rpm for 5 min. The organic layer was removed and evaporated under a gentle stream nitrogen gas at 45 °C until it was completely dry. The dried residue was dissolved with 50 μl mobile phase. After centrifugation, 5 μl of the clear supernatant was injected into the LC–MS system.

### 2.6. Method validation

#### 2.6.1. Matrix effect and extraction recovery

Three sets of calibration standard samples with seven concentrations and a blank spiked with IS were prepared for evaluation of recovery and ionization suppression or enhancement. The standard solutions were diluted with mobile phase to reach the concentrations of 0.78, 1.56, 3.13, 6.25, 12.50, 25.00, and 50.00 ng $mL^{-1}$ in set 1. Set 2 consisted of seven plasma samples spiked with standard solutions after extraction to the same concentrations as set 1. Plasma samples spiked with standard solution before extraction and blank plasma spiked with IS were processed and analyzed to obtain set 3. Three replicates of each set were used for determination of recovery and absolute matrix effect. The matrix effect (ME) and the effect of the matrix on recovery (RE) was evaluated by comparing results from analysis of three sets of samples as follows:

$$\mathrm{ME}\,(\%) = \frac{B}{A} \times 100$$

$$\mathrm{RE}\,(\%) = \frac{C}{A} \times 100$$

where *A* is the peak area of set 1, *B* the peak area of set 2, and *C* is the peak area of set 3.

#### 2.6.2. Precision, accuracy, and stability

The precision and accuracy of the assay were determined from QC samples. The intra-day precision was evaluated by repeating the analysis of the standard 5 times on the same day, and the inter-day RSD was determined by repeating the analysis on 3 consecutive days. Metoclopramide concentrations in samples were calculated using a calibration curve prepared on the same day. The stability experiments aimed at testing possible conditions in which the samples might be exposed to during sample shipping and handling. The short-term room temperature, long-term storage, stock solution, post-preparative, and freeze–thaw stabilities were tested.

## 3. Results and discussion

### 3.1. Selection of LC and MS conditions

Metoclopramide contains three nitrogen atom in the molecule which is easily protonated during ionization process to form a protonated molecular ion $[M+H]^+$ under acidic condition. In the present study, metoclopramide and prazosin (internal standard) were separately scanned with ESI and APCI positive and negative ion modes, injecting standard solutions. The base peak intensity

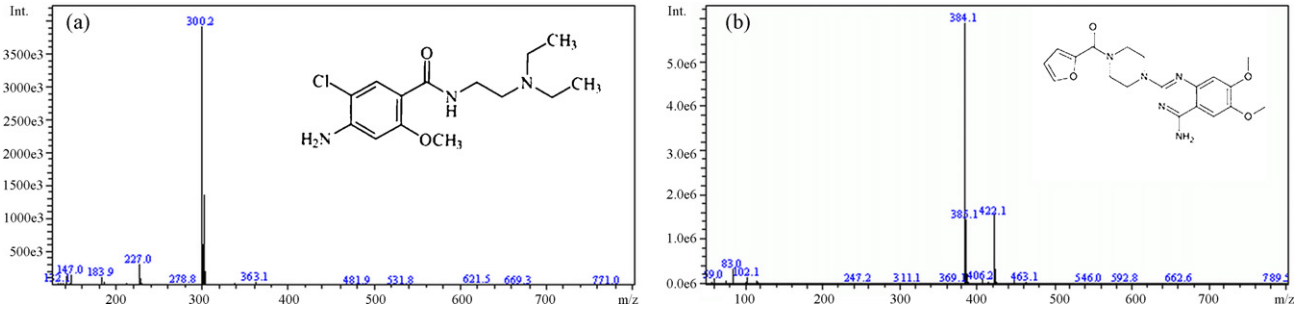


**Fig. 1.** ESI–MS positive ion scanning spectra of metoclopramide and prazosin (IS). (a) Metoclopramide and (b) prazosin.

from positive ionization was higher than those from negative ionization, and the efficiency of ionization in ESI was higher than APCI. The molecular ions with an *m*/*z* 300 $[M+H]^+$ and *m*/*z* 384 $[M+H]^+$ were produced for metoclopramide and prazosin in ESI positive ion mode. Fig. 1 shows the positive ion mass spectra of metoclopramide and prazosin, scanning from *m*/*z* 100 to 800. Selected-ion monitoring of *m*/*z* 300 for metoclopramide and *m*/*z* 384 for the IS was chosen for the method for sensitivity and selectivity. Three different types of column (Ultimate XB C18, a Shim-Pack ODS and Thermo Hypersil-Hypurity C18 column) were used to obtain optimized response, suitable retention time and good peak shapes. The Thermo Hypersil-Hypurity C18 column was selected for all analysis since it provided symmetrical peaks and gave the highest ion intensity for metoclopramide. The separation and ionization of metoclopramide and prazosin were affected by the composition of mobile phase. In the present study, 40 mM ammonium acetate (pH 3.5)–methanol–acetonitrile (75:5:20, v/v/v) was selected as an isocratic mobile phase. The retention time of metoclopramide and prazosin was <4 min. The mobile phase pH affected not only the retention time, but also the ionization efficiency. The sensitivity of metoclopramide was improved by increasing acidity of the mobile phase to pH 3.5. Raising the concentration of ammonium acetate in mobile phase increases the retention time of the analyte under the same organic solvent percentage. Thus, increasing the concentration of ammonium acetate in mobile phase required a higher percentage of organic solvent to maintain the same retention time for metoclopramide and prazosin. Increasing the proportion of methanol increases the ionization efficiency of metoclopramide and the peak shape was improved by the acetonitrile.

### 3.2. Validation of the method

#### 3.2.1. Matrix effect and extraction recovery

By comparing peak areas of samples spiked with standards after extraction with the peak areas obtained by injecting neat standard and IS directly, the extent of the absolute matrix effect was estimated (Table 1). The data presented in Table 1 indicated that the mean "absolute" matrix effects for metoclopramide and prazosin (97.2% and 96.1%, respectively) were negligible. In addition, the coefficients of variation (RSD, %) of the mean found concentration of metoclopramide at seven calibration standard concentrations in five different plasma lots were <12% (Table 2), strongly indicating little or no difference in ionization efficiency and consistent recovery of standard and internal standard from different plasma lots. Various liquid–liquid extraction methods were investigated for the extraction of metoclopramide from plasma. Comparison of extraction efficiency of different organic solvents including diethyl ether, ethyl acetate, methyl tert-butyl ether, hexane and dichloromethane, showed that ethyl acetate gave the highest recoveries (77.8% and 71.4%) for metoclopramide and the IS (Table 1) in this work. In our study, the recoveries of metoclopramide and IS were calculated by comparing the areas obtained from spiked blank plasma ($n = 5$) with those obtained from directly injecting standard solutions with the same concentrations in mobile phase.

#### 3.2.2. Selectivity

Six lots of blank plasma extracts from different sources were analyzed. Interference peaks from endogenous substances in drug-free human plasma at the retention time of metoclopramide and prazosin were not observed in any of the plasma lots. In addition, metoclopramide and prazosin were separately injected and selective ions were monitored. Fig. 2a shows one of representative chromatograms of six lots of blank plasma extracts. Fig. 2b shows a chromatogram of the plasma sample at the lowest limit of detection (0.4 ng mL$^{-1}$). Fig. 2c and d shows the chromatograms of blank plasma spiked with standard and human plasma sample after administration of metoclopramide tablets. No interference was observed.

#### 3.2.3. Sensitivity and linearity

The limit of quantitation (LOQ) using 0.4 mL plasma with acceptable accuracy and precision (< 13%) is 0.78 ng mL$^{-1}$. A good signal-to-noise ratio was observed at the LOQ. The limit of detection

**Table 1**
Extraction recovery and matrix effects of metoclopramide and prazosin ($n = 5$).

| Nominal concentration (ng mL$^{-1}$) | Mean peak area | | | ME (%) | RE (%) |
|---|---|---|---|---|---|
| | Set 1 | Set 2 | Set 3 | | |
| 0.78 | 4309 | 4294 | 3204 | 99.7 | 74.6 |
| 1.56 | 8455 | 8128 | 6682 | 96.1 | 82.2 |
| 3.13 | 15,575 | 15,395 | 10,441 | 98.8 | 67.8 |
| 6.25 | 28,973 | 28,389 | 23,587 | 98.0 | 83.1 |
| 12.5 | 48,722 | 47,423 | 38,577 | 97.3 | 81.4 |
| 25 | 85,981 | 79,654 | 65,496 | 92.6 | 82.2 |
| 50 | 151,260 | 147,608 | 108,463 | 97.6 | 73.5 |
| I.S. | 172,736 | 165,916 | 118,416 | 96.1 | 71.4 |

**Table 2**
Intermediate precision, accuracy and linear regression parameters of metoclopramide determination in human plasma ($n = 5$).

| Added concentration (ng mL$^{-1}$) | Mean measured concentration (ng mL$^{-1}$) | Imprecision (RSD, %) | Accuracy (%) |
|---|---|---|---|
| 0.78 | 0.81 | 11.2 | 103.8 |
| 1.56 | 1.58 | 9.5 | 101.3 |
| 3.13 | 3.28 | 9.4 | 104.8 |
| 6.25 | 6.14 | 8.3 | 98.2 |
| 12.5 | 12.36 | 7.7 | 98.9 |
| 25 | 25.67 | 8.3 | 102.7 |
| 50 | 48.59 | 9.3 | 97.2 |

Calibration curve: slope 0.0437; intercept 0.0715; correlation coefficient: 0.9995.

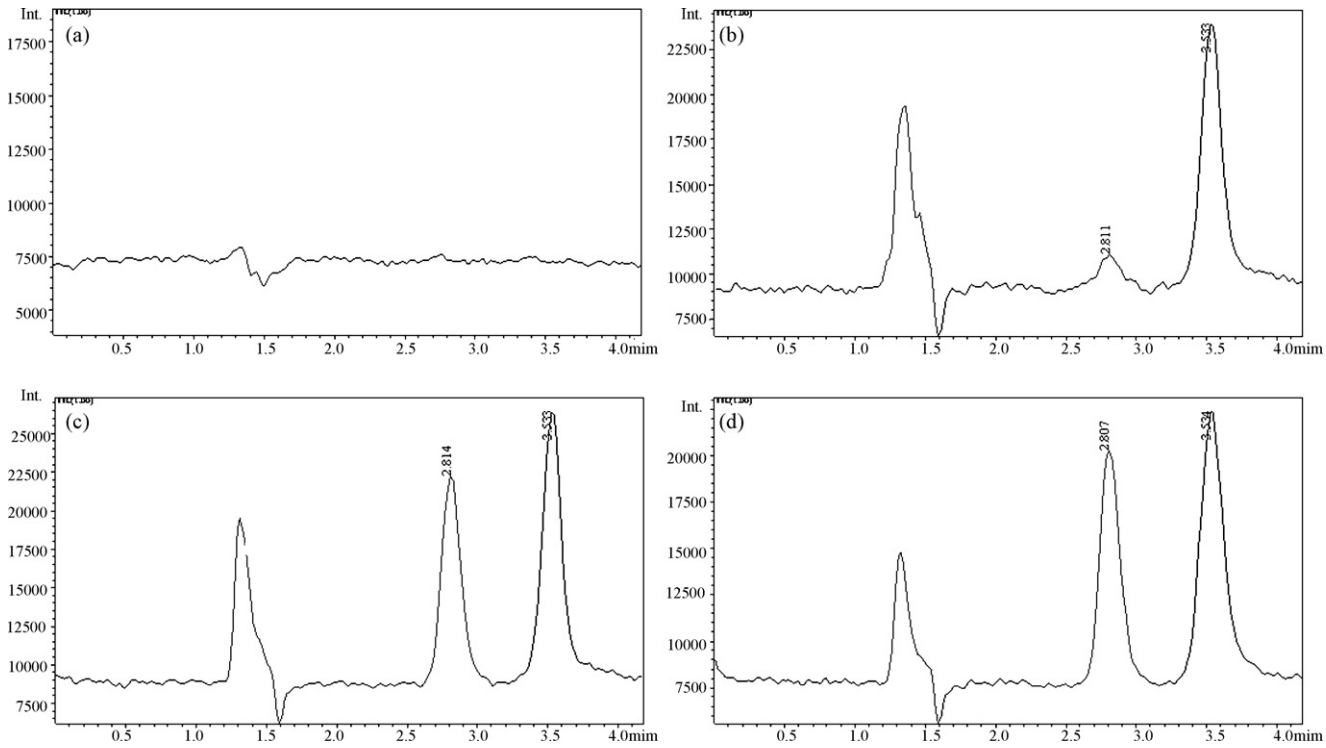


**Fig. 2.** Selective ion chromatograms of metoclopramide and prazosin (IS). (a) Blank plasma, (b) blank plasma spiked with 0.4 ng mL$^{-1}$ (the lowest limit of detection) of metoclopramide and IS, (c) blank plasma spiked with standard (25.00 ng mL$^{-1}$) and IS, and (d) human plasma sample after administration of metoclopramide (23.96 ng mL$^{-1}$) and spiked with IS. The retention time of metoclopramide and prazosin was 2.8 and 3.5 min, respectively.

(LOD) of 0.4 ng mL$^{-1}$ was estimated as the amount of metoclopramide that gave a signal 5 times the noise (S/N ≥ 5). The seven-point calibration plot obtained by least-squares regression was linear over the range 0.78–50.00 ng mL$^{-1}$ with a correlation coefficient of 0.9995 ($r^2$). The calibration curve has the regression equation $y = 0.0437x + 0.0715$, where $y$ is the peak area ratio of metoclopramide to IS and $x$ is the concentration of metoclopramide. Representative calibration curve parameters for the method from intra-day standard curve replicates are shown in Table 2. Intra-assay precision and accuracy were very satisfactory for all the concentrations tested. RSD values were less than 11.2% at all concentrations.

### 3.2.4. Repeatability, accuracy and stability

The intra- and inter-day repeatability of the method for plasma is summarized in Table 3 by analysis of replicates ($n = 5$) of LOQ and QC samples containing known concentrations of 1.56, 6.25 and 25.00 ng mL$^{-1}$ of metoclopramide. The precision of the method was described as the relative standard deviation (RSD) of each assay. The inter-day RSDs were always below 12.0% and the intra-day RSDs were within 13.6%. The accuracy of the method was evaluated by analysis the quality control samples spiked with standard solutions and was defined as the ratio of the mean computed value ($E$) to the true value ($T$) expressed as a percentage (accuracy, %). Precision and accuracy were calculated at each concentration. The results of the precision and accuracy determinations of the proposed method were acceptable for bioequivalence studies. The stability of metoclopramide and IS in human plasma under different storage conditions was evaluated as follows: QC samples were subjected to short-term room temperature conditions, to four freeze–thaw cycles stability studies, to long-term (30 days) storage conditions (−20 °C) and to processed samples kept at room temperature. All the stability studies were conducted at three concentration levels (1.56, 6.25 and 25.00 ng mL$^{-1}$) with five determinations for each. For short-term stability determination, stored plasma aliquots were thawed and kept at room temperature for a period of around 24 h during routine sample preparation. Samples were extracted and analyzed as described above. These results indicated stability under the experimental conditions of the regular analytical procedure (Table 4). Metoclopramide is stable at room temperature for at least 24 h. The analyte is also stable in human plasma when stored at −20 °C for at least 30 days and at room temperature for at least 24 h. It is also stable under the influence of four freeze–thaw cycles.

**Table 3**
Reproducibility and accuracy for metoclopramide of quality control sample in human plasma ($n = 5$).

| Nominal concentration (ng mL$^{-1}$) | Mean measured concentration (ng mL$^{-1}$) | Precision (RSD, %) | Accuracy (%) |
|---|---|---|---|
| Intra-day | | | |
| 1.56 | 1.55 | 12.0 | 99.2 |
| 6.25 | 6.26 | 7.2 | 100.2 |
| 25.00 | 25.05 | 5.0 | 100.2 |
| Inter-day | | | |
| 1.56 | 1.57 | 13.6 | 100.9 |
| 6.25 | 6.50 | 8.5 | 104.0 |
| 25.00 | 25.07 | 5.7 | 100.3 |

### 3.3. Application to bioequivalence study

The bioequivalence study was approved by the Ethical Committee of Second Xiangya Hospital of Central South University, and all subjects signed the informed consent before participation. The study was based on a single dose, randomized, 2-treatment, and 2-period cross-over design. 12 male healthy volunteers took 20-mg

**Table 4**
Stability of metoclopramide in human plasma ($n = 5$).

| Nominal concentration (ng mL$^{-1}$) | Mean measured concentration (ng mL$^{-1}$) | Precision (RSD, %) | Accuracy (%) |
|---|---|---|---|
| Short-term stability for 24 h in plasma at room temperature (RT) | | | |
| 1.56 | 1.49 | 10.43 | 95.5 |
| 6.25 | 6.31 | 11.25 | 101.0 |
| 25.00 | 24.75 | 8.46 | 99.0 |
| Four freeze–thaw cycles | | | |
| 1.56 | 1.53 | 9.67 | 98.1 |
| 6.25 | 5.96 | 8.24 | 95.4 |
| 25.00 | 25.47 | 9.34 | 101.9 |
| Storage in plasma at −20 °C for 1 month | | | |
| 1.56 | 1.46 | 10.91 | 93.6 |
| 6.25 | 5.85 | 8.38 | 93.6 |
| 25.00 | 24.63 | 6.37 | 98.5 |
| Storage in processed plasma extract at RT for 24 h | | | |
| 1.56 | 1.65 | 11.62 | 105.8 |
| 6.25 | 6.04 | 8.37 | 96.6 |
| 25.00 | 24.75 | 9.31 | 99.0 |

**Table 5**
Pharmacokinetic parameters of 20 mg single dose of metoclopramide in healthy Chinese male volunteers.

| Parameters | Mean (SD) | |
|---|---|---|
| | Test ($T$) | Reference ($R$) |
| $t_{max}$ (h) | 1.6 ± 0.8 | 1.2 ± 0.4 |
| $C_{max}$ (ng mL$^{-1}$) | 24.5 ± 4.3 | 24.4 ± 4.4 |
| $AUC_{0-24}$ (ng h mL$^{-1}$) | 184.7 ± 40.1 | 174.9 ± 36.5 |
| $AUC_{0-\infty}$ (ng h mL$^{-1}$) | 208.6 ± 49.6 | 189.1 ± 36.9 |
| $t_{1/2}$ (h) | 6.3 ± 2.0 | 5.0 ± 1.2 |

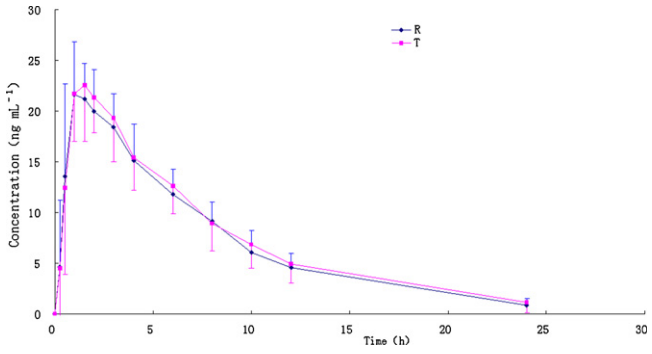


**Fig. 3.** Mean plasma concentration–time profile of metoclopramide from 12 healthy volunteers following a single oral dose of 20 mg of metoclopramide hydrochloride. $T$ – test formulation; $R$ – reference formulation.

oral dose (tablet) of metoclopramide hydrochloride with 250 mL of water. Blood samples were collected in heparinized tubes pre-dose (0 h) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h post-dose. Plasma was immediately separated by centrifugation at 4000 rpm and stored at −20 °C until analysis.

The method was successfully employed to determine metoclopramide concentrations in human plasma samples. Table 5 shows the pharmacokinetic parameters of test and reference formulations. In this study in healthy volunteers, a single, 20-mg dose of test formulation was found to be bioequivalent to reference formulation based on the rate and extent of absorption. The mean plasma concentration of metoclopramide versus time profile is presented in Fig. 3.

## 4. Conclusion

A sensitive, rapid and specific LC–MS method has been described for the determination of metoclopramide in human plasma. The limit of quantification (0.78 ng mL$^{-1}$) using 0.4 mL of plasma and a simple procedure of liquid–liquid extraction were obtained. The method has been successfully applied to bioequivalence studies. The pharmacokinetic parameters after a single 20-mg dose of metoclopramide hydrochloride in healthy volunteers were obtained.

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