

Determination of Tiapride in Human Plasma Using Hydrophilic Interaction Liquid Chromatography-Tandem Mass Spectrometry

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A rapid, sensitive and selective hydrophilic interaction liquid chromatography-tandem mass spectrometric (HILIC-MS/MS) method for the determination of tiapride in human plasma was developed. Tiapride and internal standard, metoclopramide were extracted from human plasma with dichloromethane at basic pH and analyzed on an Atlantis HILIC silica column with the mobile phase of acetonitrile-ammonium formate (190 mM, pH 3.0) (94:6, v/v). The analytes were detected using an electrospray ionization tandem mass spectrometry in the multiple-reaction-monitoring mode. The standard curve was linear ($r = 0.999$) over the concentration range of 1.00-200 ng/mL. The coefficient of variation and relative error for intra- and inter-assay at three QC levels were 6.4-8.8% and -2.0-3.6%, respectively. The recoveries of tiapride ranged from 96.3 to 97.4%, with that of metoclopramide (internal standard) being 94.2%. The lower limit of quantification for tiapride was 1.00 ng/mL using 100 μ L of plasma sample.

Key words: Tiapride, Human plasma, HILIC-MS/MS

INTRODUCTION

Tiapride (Fig. 1) is a substituted benzamide with dopamine antagonistic effects specifically on D₂ and D₃ receptors. It has been used for the treatment of various diseases, including movement disorders, emesis, migraine, chronic headache and agitation (Heun *et al.*, 2001; Kotzailias *et al.*, 2003; Robert and Allain, 2001).

Several methods for the determination of tiapride in biological fluids were reported using spectrofluorometry (Buna *et al.*, 1996), chemiluminometry (Aly *et al.*, 2001), gas chromatography (GC) with a surface ionization detector (Kamizono *et al.*, 1991) and high-performance liquid chromatography (HPLC) methods with UV (Norman *et al.*, 1986) or fluorescence detection (Chiba *et al.*, 2003). Those methods use a large amount of plasma (more than 1 mL) and do not provide good sensitivity for pharmacokinetic studies of tiapride. A sensitive, simple, fast and reliable bioanalytical method is required for human

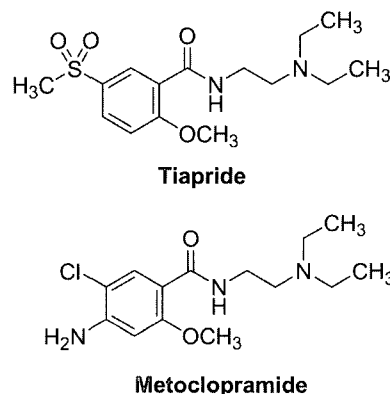


Fig. 1. Chemical structures of tiapride and metoclopramide (internal standard)

pharmacokinetics and bioequivalence studies of tiapride.

Hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) methods operated with the underivatized silica column and low aqueous-high organic mobile phase have been proved to be ideal for the analysis of polar compounds in biological fluids (Naidong, 2003; Naidong and Eerkes, 2004). The purpose of this paper was to develop and validate a HILIC-MS/MS method using liquid-liquid extraction at basic pH for the

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quantitative analysis of tiapride using 100 μ L human plasma.

MATERIALS AND METHODS

Materials and reagents

Tiapride hydrochloride and metoclopramide hydrochloride (internal standard) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and dichloromethane (HPLC grade) were obtained from Burdick & Jackson Inc. (Muskegon, MI, USA) and the other chemicals were of HPLC grade or the highest quality available. Drug-free human plasma containing sodium heparin as the anticoagulant was obtained from healthy volunteers.

Preparation of calibration standards and quality control samples

Primary stock solutions of tiapride and metoclopramide (1 mg/mL) were prepared in deionized water. Working standard solutions of tiapride were prepared by diluting each primary solution with water. The working solution for internal standard (100 ng/mL) was prepared by diluting an aliquot of stock solution with water. All tiapride and metoclopramide solutions were stored at *ca* 4°C in polypropylene bottles in the dark when not in use.

Human plasma calibration standards of tiapride (1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 100, and 200 ng/mL) were prepared by spiking appropriate amount of the working standard solutions into a pool of ten lots of drug-free human plasma. Quality control (QC) samples at 2.50, 25.0 and 150 ng/mL were prepared in bulk by adding 250 μ L of the appropriate working standard solutions (0.05, 0.5 and 3 μ g/mL) to drug-free human plasma (4750 μ L). The QC samples were aliquoted (100 μ L) into polypropylene tubes and stored -20°C until analysis.

Sample preparation

100 μ L of blank plasma, calibration standards and QC samples were mixed with 10 μ L of internal standard working solution and 100 μ L of 100 mM NaOH to adjust pH of samples to more than 11. The samples were extracted with 1000 μ L of dichloromethane in 1.5 mL-polypropylene tubes by vortex-mixing for 5 min at high speed and centrifuged at 5000 *g* for 10 min at 4°C. The organic layer was pipette transferred and evaporated to the dryness under nitrogen at 35°C. The residues were dissolved in 50 μ L of 100% acetonitrile by vortex-mixing for 2 min, transferred to injection vials, and 10 μ L were injected onto LC/MS/MS system.

LC/MS/MS analysis

For LC/MS/MS analysis, the chromatographic system

consisted of a Nanospace SI-2 pump, a SI-2 autosampler and a S-MC system controller (Shiseido, Tokyo, Japan). The separation was performed on an Atlantis HILIC Silica column (5 μ m, 3 mm i.d. \times 50 mm, Waters Co, Milford, MA, USA) using a mixture of acetonitrile-ammonium formate (190 mM, pH 3.0) (94:6, v/v) at a flow rate of 0.5 mL/min. The column and autosampler tray temperature were 40°C and 4°C, respectively. The analytical run time was 6.0 min. The eluent was introduced directly onto the tandem quadrupole mass spectrometer (Quattro LC, Micromass UK Ltd, UK) through the positive ionization electrospray interface. The ion source and desolvation temperature were held at 120°C and 350°C, respectively. The optimum cone voltages for ionization of tiapride and metoclopramide were 28 V and 28 V, respectively. Multiple-reaction-monitoring (MRM) mode using specific precursor/product ion transitions was employed for the quantification. The molecular ions of tiapride and metoclopramide were fragmented at collision energy of 20 eV and 18 eV using argon as collision gas. Detection of the ions was performed by monitoring the transitions of *m/z* 329 to *m/z* 256 for tiapride and *m/z* 300 to *m/z* 227 for metoclopramide. Peak areas for all components were automatically integrated using MassLynx Version 3.5 (Micromass UK Ltd, UK).

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 2.50, 25.0, and 150 ng/mL were assayed 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. The lower limit of quantitation (LLOQ) was set at a level where the following criteria were met: signal-to-noise ratio \geq 5.0 with RE \leq \pm 20% and CV \leq 20%.

The absolute recoveries of tiapride were determined by comparing the peak area of six extracted samples at the concentrations of 2.50, 25.0, and 150 ng/mL with the mean peak area of recovery standards. Three replicates of each of the recovery standards were prepared by adding tiapride and internal standard to blank human plasma extracts.

To evaluate the three freeze/thaw cycle stability and room temperature matrix stability, six replicates of QC samples at each of the low and high concentrations (2.50 and 150 ng/mL, respectively) were subjected to three freeze/thaw cycles or were stored at room temperature for 24 h before processing, respectively. Six replicates of QC samples at each of the low and high concentrations were

processed and stored under autosampler conditions for 24 h were assayed to assess post-preparative stability.

RESULTS AND DISCUSSION

The electrospray ionization of tiapride and metoclopramide produced the abundant protonated pseudomolecular ions (MH^+) at m/z 329 and 300, respectively under positive ionization conditions, without any evidence of fragmentation. MH^+ ions from tiapride and metoclopramide were selected as the precursor ion and subsequently fragmented in MS/MS mode to obtain the product ion spectra yielding useful structural information (Fig. 2). The fragment ions at 256 (the loss of diethylamino group from MH^+ ion) and m/z 227 (the loss of diethylamino group from MH^+ ion) were produced as the prominent product ions for tiapride and metoclopramide, respectively. The quantification of the analytes was performed using the MRM mode due to the high selectivity and sensitivity of MRM data acquisitions, where the precursor and product ions are monitored. Two pairs of MRM transitions were selected: m/z 329 \rightarrow 256 for tiapride and m/z 300 \rightarrow 227 for metoclopramide (internal standard).

Bare silica column operated with low aqueous-high organic mobile phases are viable means of analyzing polar compounds in biological fluids (Naidong, 2003). Mobile phase of high organic content would lead to favorable spraying conditions at the LC-MS interface necessary for adequate sensitivity. Tiapride was well retained on a silica column and higher acetonitrile content (94%) in the mobile phase enhanced the signal intensity of tiapride.

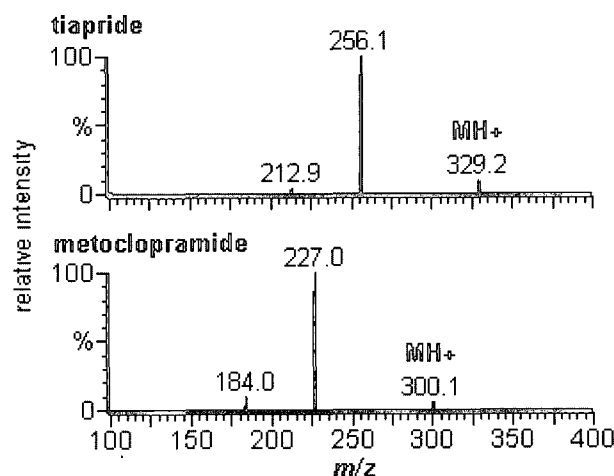


Fig. 2. Product ion mass spectra of (a) tiapride and (b) metoclopramide (internal standard).

Fig. 3 shows the representative MRM chromatograms obtained from the analysis of blank human plasma and human plasma spiked with tiapride at 1.00 ng/mL and 20.0 ng/mL. The analysis of blank human plasma samples from six different sources did not show any interference at the retention times of tiapride (4.9 min) and metoclopramide (3.3 min) (Fig. 3a), confirming the specificity of the present method.

This method was validated to meet the acceptance criteria of industrial guidance for the bioanalytical method validation (<http://www.fda.gov/cder/guidance/index.htm>, 2001). Calibration curves were obtained over the concentration range of 1.00 to 200 ng/mL of tiapride in plasma.

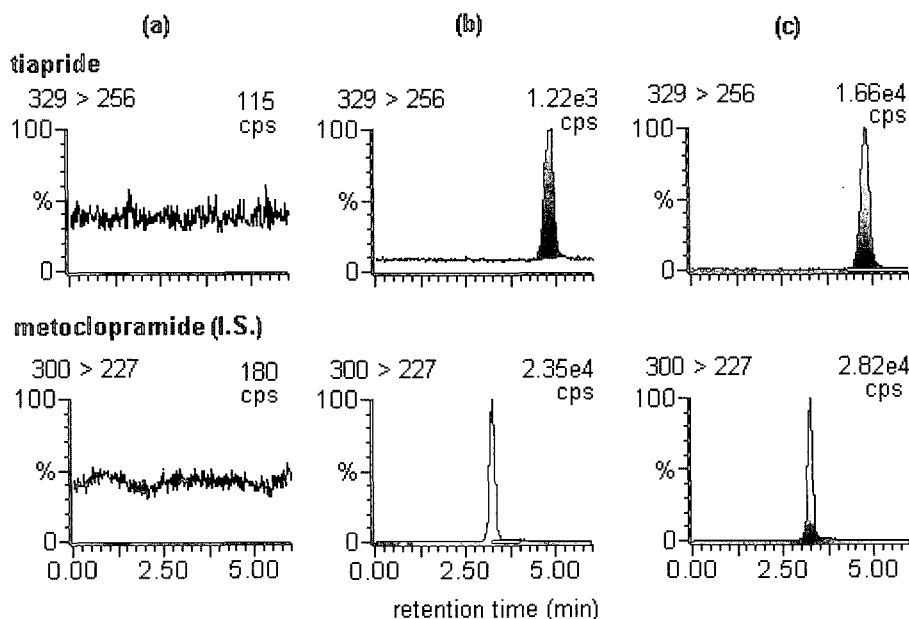


Fig. 3. MRM LC/MS/MS chromatograms of (a) a blank human plasma, (b) human plasma sample spiked with 1.00 ng/mL and (c) 20.0 ng/mL of tiapride.

Table I. Calculated concentrations of tiapride in calibration standards prepared in human plasma (n = 9)

	Theoretical concentration (ng/mL)								slope	r
	1.00	2.00	5.00	10.0	20.0	50.0	100	200		
Mean (ng/mL)	1.04	2.08	4.98	10.3	20.8	47.6	102	199	0.0530	0.999
CV (%)	7.8	4.3	5.1	9.0	4.3	5.7	6.5	4.7	10.7	
RE (%)	4.0	4.0	-0.4	3.0	4.0	-4.8	2.0	-0.5		

Linear regression analysis with a weighting of 1/concentration gave the optimum accuracy of the corresponding calculated concentrations at each level (Table I). The low coefficients of variation (CV) value for the slope indicated the repeatability of the method (Table I).

For six samples of blank plasma from six independent sources with tiapride at 2.50 ng/mL, CV and RE were 6.7% and 2.9%, respectively. These tight CV and RE values indicate no significant lot-to-lot variation in matrix effects.

Table II shows a summary of intra- and inter-batch precision and accuracy data for QC samples containing tiapride. Both intra- and inter-assay CV values ranged from 6.4 to 8.8% at three QC levels. The intra- and inter-assay RE values for tiapride were -2.0 to 3.6% at three QC levels. These results indicated that the present method has an acceptable accuracy and precision.

LLOQ was set at 1.00 ng/mL for tiapride using 100 μ L of human plasma. Representative chromatogram of an LLOQ is shown in Fig. 3b and the signal-to-noise ratio for tiapride is about 25 at 1.00 ng/mL. CV and RE at the LLOQ level were 9.8% and 2.0%, respectively (Table II).

The extraction recoveries of tiapride from spiked human plasma were determined at the concentrations of 2.50, 25.0 and 150 ng/mL in six replicates. The recoveries of tiapride ranged from 96.3 to 97.4%, with that of metoclopramide (internal standard) being 94.2 \pm 7.4% (Table III). The one-step liquid-liquid extraction with dichloromethane at pH 11 has been successfully applied to the extraction of tiapride from human plasma.

Stability of tiapride during sample handling (freeze-thaw and short-term temperature stability) and the stability of processed samples were evaluated (Table IV). Three freeze-thaw cycles and room temperature storage of the

Table II. Precision and accuracy of tiapride in quality control samples

QC (ng/mL)	Intra-batch (n=6)				Inter-batch (n=18)			
	1.00	2.50	25.0	150	2.50	25.0	150	
Mean (ng/mL)	1.02	2.47	25.6	151	2.45	25.9	152	
CV (%)	9.8	7.0	7.4	8.8	7.5	7.4	6.4	
RE (%)	2.0	-1.2	2.4	0.7	-2.0	3.6	1.3	

QC samples for 24 h before analysis had little effect on the quantification. Extracted QCs and calibration standards were allowed to stand at ambient temperature for 24 h prior to injection without affecting the quantification.

In conclusion, a sensitive and reliable LC/MS/MS method for the analysis of tiapride in human plasma using HILIC has been successfully developed and validated. To extract tiapride in the plasma, a liquid-liquid extraction with dichloromethane after alkaline treatment to pH 11 was used. The LLOQ for tiapride was 1.00 ng/mL using 100 μ L of plasma. The method may be suitable for the clinical pharmacokinetic study of tiapride.

Table III. Absolute recoveries of tiapride and metoclopramide (internal standard) from spiked human plasma

Concentration (ng/mL)	Recovery (%; mean \pm SD, n=6)	
	Tiapride	metoclopramide
2.50	97.2 \pm 8.2	–
25.0	97.4 \pm 5.6	–
150	96.3 \pm 7.8	–
5.00	–	94.2 \pm 7.4

– : Not assayed

Table IV. Stability of samples (n=6).

Statistical variable	Theoretical concentration (ng/mL)	
	2.50	150
freeze and thaw stability		
Mean	2.53	154
CV (%)	4.1	7.2
RE (%)	1.2	2.7
Short-term temperature stability (24 h at room temperature)		
Mean	2.46	154
CV (%)	6.1	4.2
RE (%)	1.6	2.7
Post-preparative stability (24 h at room temperature)		
Mean	2.49	151
CV (%)	6.4	6.9
RE (%)	0.4	0.7

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