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Full Length Research Paper

A sensitive, fast and accurate liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–MS/MS) method for the pharmacokinetic study of cyclobenzaprine tablets

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A simple, fast and accurate liquid chromatography–electrospray ionization-tandem mass spectrometry method was developed and validated for the quantification of cyclobenzaprine in human plasma. After a simple single-step liquid–liquid extraction with ethyl acetate, the analyte was separated in an Intersil ODS-3 column through isocratic elution with methanol–water containing 0.1% formic acid (80:20, v/v) at a flow rate of 0.2 ml/min and analyzed by mass spectrometry in the positive ion MRM mode. Good linearity was achieved from 0.04 to 50 ng/ml. The intra- and interday precisions were less than 12.1%, and accuracy ranged from 99.6 to 106.2%. The elimination half-lives ($t_{1/2\,z}$) after a single oral administration of 5, 10, and 15 mg cyclobenzaprine tablets were 25.5, 26.2, and 26.4 h, respectively. The means of C_{max} and AUC increased proportionally to the cyclobenzaprine doses. The pharmacokinetics of cyclobenzaprine fit the linear dynamics of the cyclobenzaprine dose range in healthy Chinese volunteers.

Key words: Cyclobenzaprine, liquid chromatography, electrospray ionization, tandem mass spectrometry.

INTRODUCTION

Cyclobenzaprine,-(5H-dibenzo[a,d]cyclohepten-5-ylidene) -N,N- dimethyl- 1-propanamine (Figure 1), was first introduced in 1977 and is a widely prescribed skeletal muscle relaxant with proven efficacy and safety profile in the treatment of acute muscle spasm (Katz and Dube, 1988; Browning et al., 2001; Borenstein and Korn 2003; Toth and Urtis, 2004; Weil et al., 2010; Malanga et al., 2009), acute cervical strain (Khwaja et al., 2010), and myofascial pain (Leite et al., 2009) in occidental populations.Various methods such as high performance liquid chromatography (HPLC) with ultraviolet (UV) spectrophotometry detector (Hwang et al., 1993; Constanzer et al., 1995) and gas chromatography (GC) (Hucker et al., 1977) have been developed for the quantification of cyclobenzaprine in biological samples. However, these methods have several limitations including long run-time, poor reproducibility, and inadequate sensitivity. Liquid chromatography, coupled with mass spectrometry (LC/MS) techniques, has recently been widely used in analyzing drug compounds in biological fluids because of its excellent specificity, and sensitivity. LC/MS using different modes of ionization has been reported to detect cyclobenzaprine in plasma and urine (Darwish et al., 2008; Darwish and Xie, 2009; Darwish et al., 2009; Coulter et al., 2010; Darwish and Hellriegel, 2011; Gai et al., 2009; Winchell et al., 2002). However, the application of this method is limited to clinical pharmacokinetics because of several disadvantages such as low sensitivity, costly, complicated, and labor- intensive sample preparation procedures as well as large sample volume consumption. Thus, a

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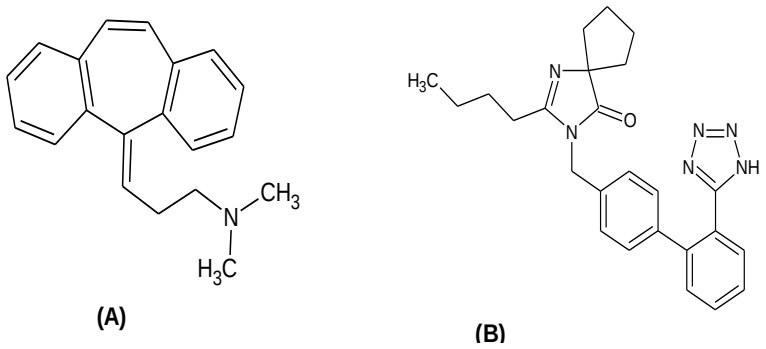

Figure 1. Chemical structures of Cyclobenzaprine (A) and Irbesartan (B).

single-step extraction, a run-time of 4.2 min, a more sensitive LOQ of 0.04 ng/ml and specific analytical method to determine cyclobenzaprine in human plasma was developed and applied to the pharmacokinetic investigation of a single oral administration of three doses (5, 10, and 15 mg) of cyclobenzaprine tablets in healthy Chinese subjects.

MATERIALS AND METHODS

Chemicals and reagents

Standard (purity >99.5%) and test drug tablets (5 and 10 mg) of cyclobenzaprine was provided by the Yunnan Longhai Natural Botanical Pharmaceutical Co., Ltd. (Yunnan, China). Irbesartan (Figure 1, purity >99.0 %), as the internal standard (IS), was provided by Jiangsu Wangao Pharmaceutical Co., Ltd. (Jiangsu, China). methanol (Merck, Germany) and formic acid (DIMA, USA) were of HPLC grade. Acetic ether of analytical grade was commercially available. The water used in the experiment was distilled twice in the laboratory.

LC–MS/MS instrument and conditions

HPLC was performed using a Finnigan Surveyor LC Pump Plus equipped with a Finnigan Surveyor Autosampler Plus (Thermo Electron Corporation, USA). Room temperature was controlled at 20°C by an air conditioner. Chromatographic separation was performed in an Intersil ODS-3 column (150 × 4.6 mm i.d., 5 µm, GL Sciences, Japan) with a SecurityGuard C18 column (4 × 2.0 mm i.d., Phenomenex, USA) at room temperature. An isocratic elution mode was adopted with a mobile phase consisting of methanol–water with 0.1% formic acid (80:20, v/v) at a flow rate of 0.2 ml/min. A flow-switching technique was adopted by controlling the flow channel selection valve. The liquid was only loaded to the MS when the target peaks emerge to prevent redundant co-elutes and interferences from polluting the analyzer and increasing the MS signals of the targets. Mass spectrometric detection was performed in a Finnigan TSQ Quantum Discovery MAX system (Thermo Electron Corporation, USA) with an ESI interface operating at the positive ionization mode. The optimized MS parameters were as follows: Spray voltage, 4.6 kV; capillary temperature, 300°C; source CID, 10 V; sheath gas pressure, 35 Arb; and auxilliary gas pressure, 3 Arb. The detector was operated at unit resolution in multiple reaction monitoring (MRM) modes, with a scan time of 0.5 s per transition. The optimized fragmentation transitions for MRM were *m*/*z* 276.1→216.0 for cyclobenzaprine and *m*/*z* 429.2→207.0 for IS. The collision energy was set at 33 V for cyclobenzaprine and 25 V for IS. Data processing was performed with the Xcalibur workstation software (Ver. 1.4).

Preparation of standard and quality control (QC) samples

The cyclobenzaprine primary stock solution (1.0 mg/ml) was prepared by dissolving accurately weighed reference compounds in methanol. A 46.7 ng/ml working solution of IS was prepared by further diluting the IS stock solution with methanol. All these solutions were stored at 4°C and brought to room temperature before use.

The calibration samples cyclobenzaprine concentrations were prepared by spiking appropriate amounts of the working solution in blank human plasma. The calibration curves were prepared, and the QC samples and each batch of clinical plasma samples were assayed together. The QC samples were prepared at three different concentration levels of low, mid, and high. The QC samples were prepared in drug-free human plasma from a second set of cyclobenzaprine working solutions. These solutions were prepared using different weights of the analyte standard.

Sample preparation

Vials containing frozen plasma samples were placed in 37°C water to thaw. Plasma (200 µl) and IS working solution (10 µl) were added to a 2 ml plastic conical extraction tube. Ethyl acetate (1 ml) was added after vortex-mixing for 30 s. The tube was covered well and vigorously shaken for 3 min. After centrifugation at 14,000×*g* for 10 min, the upper organic layer was transferred to another plastic tube and evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was reconstituted in 100 µl mobile phase and centrifuged at 14 000×*g* for 10 min. The supernatant was pipetted to an autosampler vial, and 10 µl of the supernatant was injected into the column for analysis.

Method validation

The proposed method was validated using the plasma samples according to the FDA Guidance for Industry, Bioanalytical Method Validation (US, 2001), the limit of detection (LOD) and quantification

(LLOQ), accuracy precision, recovery and stability. The LOD and LOQ were determined as the lowest concentration giving a response of three times and 10 times, respectively, the average of the base line noise defined from six drug-free samples. Linearity was assessed using weighted ($1/x^2$) least squares linear regression of calibration curves generated in triplicate on three consecutive days using the ratio of analyte to I.S. The acceptance criterion for a calibration curve was a correlation coefficient (*r*) of 0.99 or better, with each back-calculated standard concentration within 15% deviation from the nominal value, except at the limit of quantitation (LOQ), in which the maximum acceptable deviation was set at 20%.The intra- and interday precisions (RSD) and accuracies were determined by analyzing six replicate QC samples on three different days, whereas the inverse prediction of concentrations was determined from the calibration curve. The acceptance criteria for each back-calculated concentration were precision <15% and accuracy <15% of the nominal value. The recoveries were estimated by comparing the peak areas of cyclobenzaprine in three replicates of QC samples with those of post-extraction blank matrix extracts at the corresponding concentrations. The matrix effects of cyclobenzaprine were evaluated by comparing the peak areas of post-extraction blank plasma that were spiked at certain concentrations of QC samples with the areas obtained by the direct injection of the corresponding standard solutions. The stability of cyclobenzaprine in the plasma samples was determined from three QC levels with three replicates each under the following conditions: Long-term stability at -20°C for 59 days, short-term stability at 25°C for 5 h, using processed samples in autosampler vials for 24 h, and after three freeze/thaw cycles (-20 to 25°C).

Pharmacokinetic study

The single-dose pharmacokinetics of cyclobenzaprine tablets was studied in healthy Chinese subjects, according to the Declaration of Helsinki and Good Clinical Practice. The study protocol was approved by the Medical Ethical Committee of the First Affiliated Hospital of Bengbu Medical College (Anhui, China). Thirty healthy male and female subjects (19 to 24 years old, 46 to 69.5 kg body weights) were enrolled and participated in the study after signing a consent form. The subjects had no history of cardiovascular, hepatic, renal, gastrointestinal, hematologic, nervous, or any acute or chronic diseases or drug allergy and had stopped using any drug 2 weeks prior to the study. Physical examination and laboratory tests showed no abnormal findings. All subjects were randomized into 5, 10, and 15 mg dose groups (five males and five females in each group). After fasting overnight, each subject was orally administered with the corresponding doses. Water intake was allowed 2 h post-dose, and standard meals were provided at 4 and 10 h post-dose. The subjects were required to refrain from smoking, alcohol, caffeine, and strenuous exercise during the study and under direct medical supervision at the study site. Blood samples (3 ml) were collected in heparinized tubes at 0 h (pre-dose) and 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 168 and 192 h post-dose. After centrifugation, plasma was separated and stored at -80°C until analysis.

RESULTS AND DISCUSSION

Method development

Full scans were performed in the positive ion detection mode to develop the ESI conditions for cyclobenzaprine and IS. Cyclobenzaprine and IS predominantly formed protonated molecular ions $[M+H]^+$ at m/z 276.1 and m/z 429.2, respectively, in the Q1 full scan MS (Figure 2). Cyclobenzaprine and IS gave the most abundant fragment ion at m/z 216.0 and 207.0, respectively. Thus, the mass transitions that were chosen for quantitation were *m*/*z* 276.1→216.0 for cyclobenzaprine and *m*/*z* 429.2→207.0 for IS. The MRM mode was chosen for assay development, providing higher sensitivity and selectivity than the reported methods (Darwish et al., 2008; Darwish and Xie, 2009; Darwish et al., 2009; Coulter et al., 2010; Darwish and Hellriegel, 2011; Gai et al., 2009; Winchell et al., 2002).

The chromatographic conditions, especially the composition of the mobile phase, were optimized through several trials to achieve good resolution, sensitivity, and symmetric peak shapes for cyclobenzaprine and IS. Different percentages of methanol–water solutions containing 0.1% formic acid were tested considering that the formic acid solution in the mobile phase can aid in ionizing the analytes, enhancing ion response, and modifying the peak shape. Finally, a methanol–water solution containing 0.1% formic acid (80:20, v/v) was adopted as the mobile phase because of its better separation, high sensitivity, and more stable MS signal. Cyclobenzaprine and IS were detected at retention times of 2.9 and 4.2 min, respectively, using the optimized LC–MS/MS conditions and not interfered by endogenous compounds. The matrix effects were also evaluated by comparing the peak areas of cyclobenzaprine from the spike after extraction of the samples (the blank plasma samples were obtained from six different sources) to those obtained for the standards in the mobile phase at equivalent concentrations. The ratios, from low to high dose levels, were 88.2 ± 3.2, 88.5 ± 2.8, and 91.4 ± 1.8%. The same assay was performed for the IS, and the ratio was 93.6 ± 3.5%. These results indicate that the matrix effect should not have a significant impact on assay performance. Choosing the appropriate IS is important to achieve high accuracy and to deal with sample matrix effects when LC–MS/MS is used for the assay. Irbesartan was selected as the IS because of its chromatographic behavior similar to that of cyclobenzaprine but with better extraction efficiency compared with diazepam, chlorobenzene, chlorzoxazone, naproxen, and so on.

Specificity

The specificity of the proposed method was evaluated by analyzing individual blank plasma samples from six different sources. All samples had no interference from endogenous substances at the retention time of either the analyte or the IS. Typical chromatograms of a blank plasma, a spiked plasma sample with cyclobenzaprine and IS, and a plasma sample from a subject are shown in Figure 3

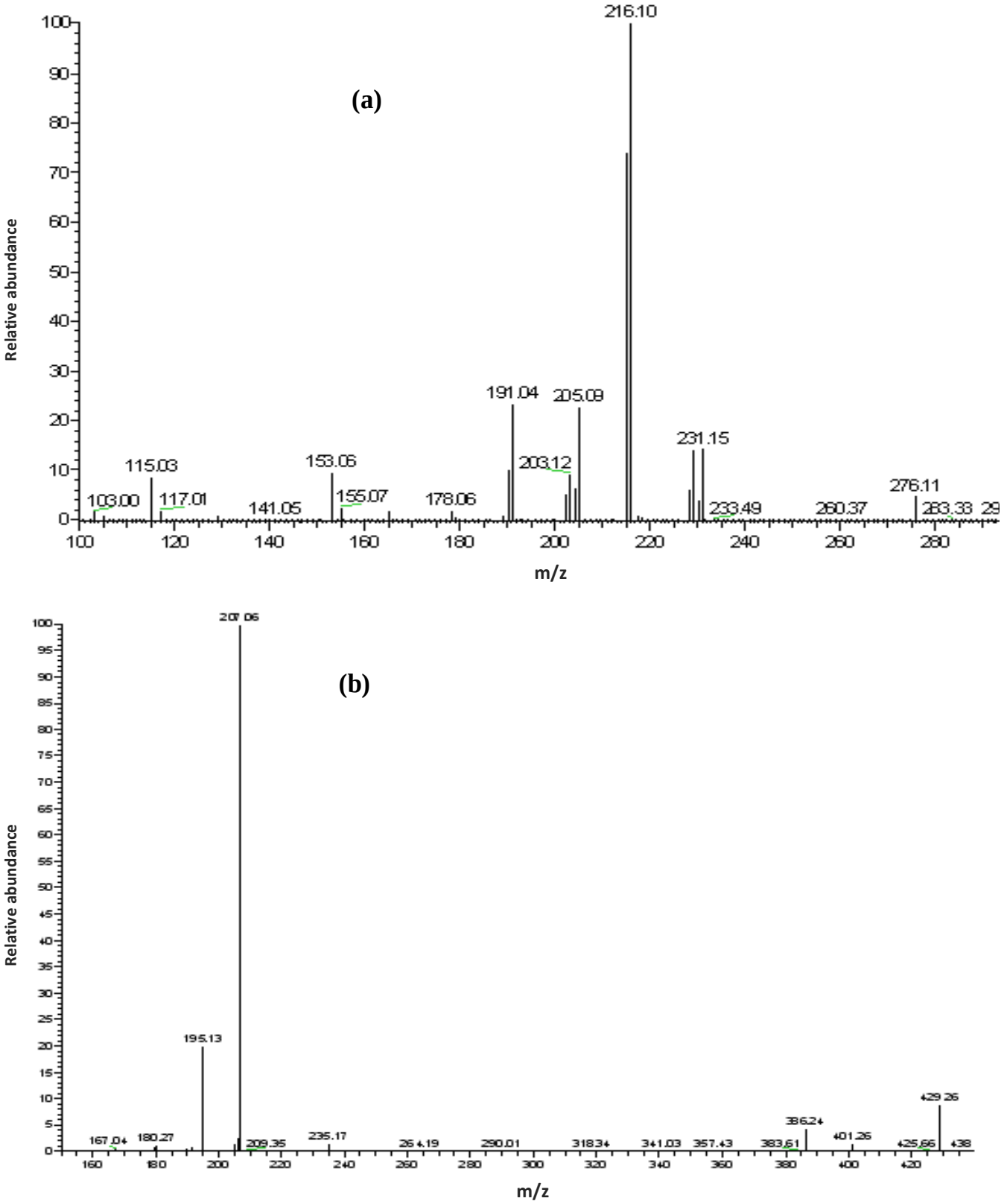

Figure 2. Product ion mass spectra of the $[M+H]^+$ ions of (A) cyclobenzaprine and (B) IS.

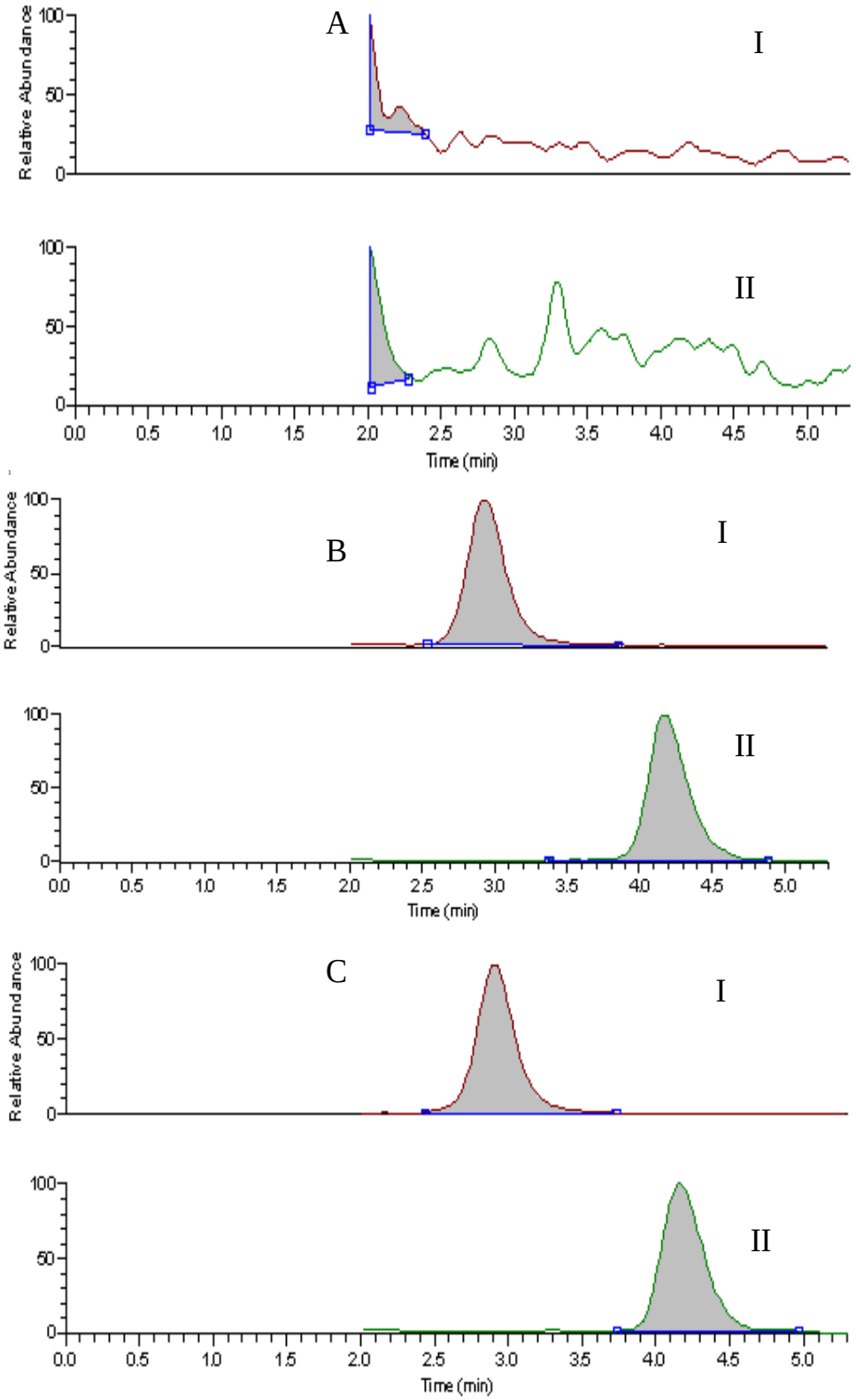

Figure 3. Representative MRM chromatograms of (A) blank human plasma; (B) blank plasma spiked with cyclobenzaprine and IS; (C) plasma obtained from a subject 3 h after orally administration of 5 mg cyclobenzaprine tablet. (I) Cyclobenzaprine (*m/z* 276.1→216.0) and (II) IS (*m/z* 429.2→207.0).

Table 1. Accuracy and precision for the analysis of cyclobenzaprine in QC samples (*n* = 3 days, six replicates per day).

Spiked concentration (ng/ml)	Intra-day(First day)			Inter-day		
	Measured concentration (ng/ml)	R.S.D (%)	Accuracy (%)	Measured concentration (ng/ml)	R.S.D (%)	Accuracy (%)
0.1	0.098 ± 0.005	5.0	106.0	0.100±0.010	10.0	99.9
2	2.13 ± 0.11	5.3	106.2	2.05±0.12	6.1	102.6
30	29.89 ± 3.62	12.1	99.6	30.26±2.44	8.1	100.9

LOD, LOQ, calibration curve and linearity

The LOD and LOQ for cyclobenzaprine were 0.012 and 0.04 ng/ml, respectively, which is superior to the reported methods (LLOQ, 0.1 ng) by using the atmospheric pressure chemical ionization (APCI; Mallet et al., 2004). The calibration curve was constructed by plotting the peak area ratios (*y*) of cyclobenzaprine to IS versus the plasma concentrations (*x*) of cyclobenzaprine. Good linearity was exhibited over the concentration range of 0.04 to 50 ng/ml with correlation coefficient $r > 0.994$. A typical equation of the calibration curve is $y = (0.5808)x+0.0936$, where $r = 0.9987$, which was sensitive enough for the pharmacokinetic study of cyclobenzaprine until 192 h (12 h, Gregory et al., 2002) after a single oral dose of 5 mg cyclobenzaprine table in humans. The precision (10.5%) and accuracy (91.6%) at this concentration level were acceptable.

Accuracy and precision

The intra- and interday precision and accuracy of the assay were investigated by analyzing the QC samples (0.1, 2 and 30 ng/ml), and all the results are shown in Table 1. The intraday RSD was below 12.1% with an accuracy range of 99.6 to 106.2%. By contrast, the interday RSD was below 10.0% with an accuracy range of 99.9 to 102.6%. The method was proven to be highly accurate and precise (Table 1).

Recovery and stability

Protein precipitation (such as acetonitrile or methanol) as sample preparation always reduces the MS sensitive with an increased number of subsequent injections. Moreover, if deproteinization is not through, the impurity in sample liquid might block the LC-MS pipelines. In addition, for avoiding the high cost of SPE (Gai et al., 2009; Winchell et al., 2002), Constanzer et al. (1995) used hexane as the extraction solvent for the compound from basified biological sample, but the extraction procedure was labor-intensive and consumed more extraction solvent (5 ml) and samples (1 ml). Different liquid–liquid extraction (LLE) conditions were evaluated including different pH and organic extraction solvents. Cyclobenzaprine could be extracted with diethyl ether, ethyl acetate, and dichloromethane. Among them, extraction efficiency with ethyl acetate was better satisfied. Moreover, IS also has a good recovery under the current condition. The plasma sample was extracted only once (single-step extraction) in the proposed method using only 1 ml of the extraction solvent and 0.2 ml of the sample during the single extraction procedure. This modification significantly simplified the sample preparation procedure and also met the required recovery for the assay. The extraction recoveries of cyclobenzaprine were 83.3 ± 4.1, 83.3 ± 6.3 and 84.6 ± 3.2% at concentrations of 0.1, 2, and 30 ng/ml, respectively, whereas that of IS was 90.1 ± 3.5%. These results suggest that the recoveries of cyclobenzaprine and IS are consistent and not concentration-dependent.

The stability of cyclobenzaprine in the plasma, under different conditions, was investigated. Cyclobenzaprine was found to be stable after three freeze–thaw cycles, at ambient temperature for 5 h in plasma, in the processed samples in the autosampler vials for 24 h. The compound was stored at -20°C for 59 days. All decompositions under this condition were below 15% compared with those samples that were analyzed immediately (Table 2). Hence, cyclobenzaprine can be stored and extracted under routine laboratory conditions without special attention.

Pharmacokinetic study

After a single oral administration of 5, 10, and 15 mg cyclobenzaprine tablets, the plasma cyclobenzaprine concentrations were successfully determined using the proposed LC–MS/MS method. The mean plasma concentration–time curves of cyclobenzaprine are shown in Figure 4. The major pharmacokinetic parameters are reported in Table 3. The maximum plasma concentrations were 5.0±2.0 μg L^{-1}(3.1-9.8), 7.2±3.2 μg L^{-1}(3.6-14.1), 17.2±8.8 μg L^{-1}(5.9-36.3) at 5.1±2.8, 5.3±1.9 and 4.0±1.8 h, respectively. The sharp fluctuant of plasma concentration range remind clinical staffs to attention personalized medicine during treatment with cyclobenzaprine. The major pharmacokinetic parameters in Chinese healthy volunteers were similar to those

Table 2. Stability of cyclobenzaprine in the plasma samples (*n* = 3 days).

Condition	Concentration (ng/ml)			Ratio of decompositions (%)		
	Low	Middle	High	Low	Middle	High
Room temperature, 0 h	0.098	1.932	28.457			
	0.099	2.122	28.672			
	0.098	2.036	28.723			
Room temperature, 5 h	0.097	1.953	29.774	-1.36	-3.79	4.04
	0.091	2.021	30.32	-7.46	-0.44	5.95
	0.101	1.92	30.106	2.71	-5.42	5.20
Room temperature, 24 h	0.089	1.976	29.573	-9.49	-2.66	3.34
	0.103	1.917	29.094	4.75	-5.57	1.67
	0.088	2.026	30.859	-10.51	-0.20	7.83
Three freeze–thaw cycles	0.095	1.997	27.992	-3.39	-1.63	-2.19
	0.092	2.058	26.965	-6.44	1.38	-5.77
	0.085	2.153	28.819	-13.56	6.06	0.70
−20°C, 59 day	0.107	2.041	27.278	8.81	0.54	-4.68
	0.101	2.71	31.925	-6.16	1.905	11.56
	0.087	2.179	30.327	7.34	-11.53	5.97

Ratio of decompositions = (being analyzed late -being analyzed immediately)/ being analyzed immediately.

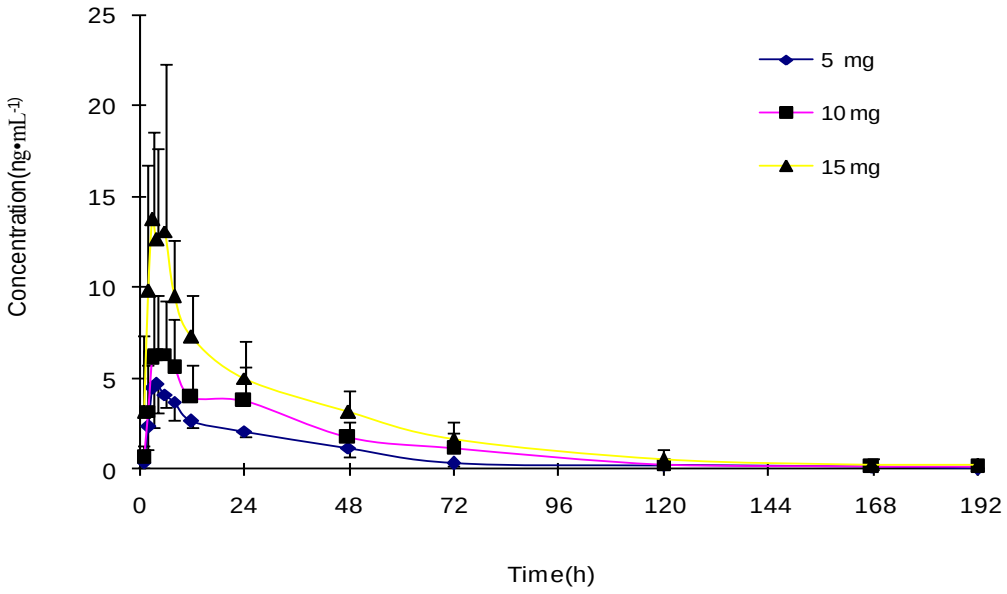

Figure 4. Mean plasma concentration–time curves of cyclobenzaprine in Chinese healthy subjects following single dose of cyclobenzaprine tablets, 5 (*n* = 9), 10(*n* = 9) and 15 mg (*n* =10, mean ± S.D.).

reported in American healthy volunteers (Winchell et al., 2002) at same doses except $t_{1/2Z}$ (25 h *vs* 18 h). The means of C_{max} and AUC increased proportionally to the cyclobenzaprine doses. The mean values of $t_{1/2Z}$

Table 3. Main pharmacokinetic parameters of cyclobenzaprine in Chinese healthy subjects following single dose of cyclobenzaprine tablets 5 (n = 9), 10 (n = 9) and 15 mg (n = 10), (mean±S.D.).

Parameter	5 mg	10 mg	15 mg
t_{max}(h)	5.1 ±2.8	5.3 ±1.9	4.0 ±1.8
C_{max} (µg L^{-1})	5.0 ±2.0	7.2 ±3.2	17.2 ±8.8
$t_{1/2z}$ (h)	25.5 ±9.5	26.2 ±5.2	26.4 ±9.1
$MRT_{(0-t)}$ (h)	29.9 ±6.9	36.3 ±7.0	35.1 ±8.9
CLz (L h^{-1})	36.5 ±5.9	47.6 ±16.6	43.5 ±22.6
Vz (L)	1292.6 ±361.2	1725.3 ±506.0	1501.7 ±512.3
$AUC_{(0-t)}$ (µg h L^{-1})	135.9 ±24.0	245.2 ±134.2	414.2 ±171.4
$AUC_{(0-\infty)}$ (µg hL^{-1})	140.4 ±24.7	248.3 ±136.7	421.7 ±179.6

apparently depended on the dose and ranged from 25.5 to 26.4 h, indicating that cyclobenzaprine was gradually cleared from the plasma. The MRT values were similar. No significant difference (p > 0.05) among the three groups, as well as between Vz and CLz, was observed. These results indicate that the pharmacokinetics of cyclobenzaprine fit the linear dynamic features of 5 to 15 mg orally administered in healthy Chinese volunteers.

Conclusion

The optimized LC–MS/MS method was validated to guarantee a reliable determination of cyclobenzaprine in human plasma. Good linearity was observed from 0.04 to 50 ng/ml. The proposed method is suitable for related pharmacokinetic studies because of its simplified sample pretreatment, high sensitivity, selectivity, precision, accuracy, and short retention time. The method was successfully applied in the determination of cyclobenzaprine in human plasma. The pharmacokinetic profiles of cyclobenzaprine were investigated for the first time in healthy Chinese subjects after single oral administration of 5, 10, and 15 mg cyclobenzaprine tablets.

REFERENCES

Borenstein DG, Korn S (2003). Efficacy of a low-dose regimen of cyclobenzaprine hydrochloride in acute skeletal muscle spasm: results of two placebo-controlled trials. Clin. Ther., 25: 1056-1073.

Browning R, Jackson JL, O'Malley PG (2001). Cyclobenzaprine and back pain: a meta-analysis. Arch. Int. Med., 161: 1613-1620.

Constanzer M, Chavez C, Matuszewski B (1995). Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine. J. Chromatogr. B, 666: 117-126.

Coulter C, Taruc M, Tuyay J, Moore C (2010). Antidepressant drugs in oral fluid using liquid chromatography-tandem mass spectrometry. J. Anal. Toxicol., 34: 64-72.

Darwish M, Chang S, Hellriegel ET (2009). A pharmacokinetic comparison of single doses of once-daily cyclobenzaprine extended-release 15 and 30 mg: A randomized, double-blind, two-period crossover study in healthy volunteers. Clin. Ther., 31: 108-114.

Darwish M, Hellriegel ET (2011). Steady-state pharmacokinetics of once-daily cyclobenzaprine extended release: A randomized, double-blind, 2-period crossover study in healthy volunteers. Clin. Ther., 33: 746-753.

Darwish M, Hellriegel ET, Xie F (2008). Single-Dose Pharmacokinetics of Once-Daily Cyclobenzaprine Extended Release 30 mg versus Cyclobenzaprine Immediate Release 10 mg Three Times Daily in Healthy Young Adults. Clin. Drug Invest., 28: 793-801.

Darwish M, Xie F (2009). Comparison of the single-dose pharmacokinetics of once-daily cyclobenzaprine extended-release 30 mg and cyclobenzaprine immediate-release 10 mg three times daily in the elderly: a randomized, open-label, crossover study. Drugs Aging, 26: 95-101.

Darwish M, Xie F (2009). Effect of food on the pharmacokinetics of once-daily cyclobenzaprine extended-release 30 mg: a randomized, open-label, crossover, single-centre study. Clin. Drug Investig., 29: 145-152.

Gai MN, Costa E, Arancibia A (2009). Bioavailability of a controlled-release cyclobenzaprine tablet and influence of a high fat meal on bioavailability. Int. J. Clin. Pharm. Ther., 47: 269-274.

Gregory A, Winchell PhD, Joyce D, King MS, Cynthia M, Chavez-Eng MS, Marvin L, Constanzer BS, Scott H, Korn MD (2002). Cyclobenzaprine Pharmacokinetics,Including the Effects of Age, Gender,and Hepatic Insufficiency. J. Clin. Pharm., 42: 61-69.

Hucker HB, Stauffer SC, Albert KS, Lei BW (1977). Plasma levels and bioavailability of cyclobenzaprine in human subjects. J. Clin. Pharm., 17: 719-727.

Hwang P, Young D, Straughn A, Meyer M, Quantitative J (1993). Determination of Cyclobenzaprine in Human Plasma by High Pressure Liquid Chromatography. Liq. Chromatogr., 16: 1163-1171.

Katz WA, Dube J (1988). Cyclobenzaprine in the treatment of acute muscle spasm: review of a decade of clinical experience. Clin. Ther., 10: 28- 216.

Khwaja SM, Minnerop M, Singer AJ (2010). Comparison of ibuprofen, cyclobenzaprine or both in patients with acute cervical strain: a randomized controlled trial. CJEM, 12: 39-44.

Leite FM, Atallah AN, El Dib R, Grossmann E, Januzzi E, Andriolo RB, da Silva EM (2009). Cyclobenzaprine for the treatment of myofascial pain in adults. Cochrane Database Syst. Rev., 8: CD006830.

Malanga GA, Ruoff GE, Weil AJ, Altman CA, Xie F, Borenstein DG (2009). Cyclobenzaprine ER for muscle spasm associated with low back and neck pain: two randomized, double-blind, placebo-controlled studies of identical design. Curr. Med. Res. Opin., 25: 1179-1196.

Toth PP, Urtis J (2004).Commonly used muscle relaxant therapies for acute low back pain: A review of carisoprodol, cyclobenzaprine hydrochloride, and metaxalone. Clin. Ther., 26: 1355-1367.

US (2001).Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, Centre for Drug Evaluation and Research, CDER, Rockville.

Weil AJ, Ruoff GE, Nalamachu S, Altman CA, Xie F, Taylor DR (2010). Efficacy and tolerability of cyclobenzaprine extended release for acute muscle spasm: a pooled analysis. Postgrad. Med., 122: 158-169.

Winchell GA, King JD, Chavez-Eng CM, Constanzer ML, Korn SH (2002). Cyclobenzaprine pharmacokinetics, including the effects of age, gender, and hepatic insufficiency. J. Clin. Pharm., 42: 61-69.