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High performance liquid chromatography-electrospray ionization mass spectrometric determination of tolterodine tartrate in human plasma

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Abstract

A selective and sensitive high performance liquid chromatography-electrospray ionization mass spectrometry method has been developed for the determination of tolterodine tartrate in human plasma. With oxybutynin as internal standard, tolterodine tartrate was extracted from plasma with *n*-hexane: isopropanol (95:5, v/v). The organic layer was evaporated and the residue was redissolved in mobile phase comprised of acetonitrile–water (10 mM CH_3COONH_4, pH 3.0) = 50:50 (v/v). An aliquot of 10 μl was chromatographically analyzed on a prepacked Shimadzu Shim-pack VP-ODS C_{18} column (150 mm × 2.0 mm I.D.) by means of selected-ion monitoring (SIM) mode mass spectrometry. Standard curves were linear ($r = 0.9993$) over the concentration range of 0.1–30.0 ng/ml and had good accuracy and precision. The within- and between-batch precisions were within 10% relative standard deviation. The limit of detection (LOD) was 0.05 ng/ml. The validated LC–ESI–MS method has been used successfully to study tolterodine tartrate pharmacokinetic, bioavailability and bioequivalence in 20 healthy male volunteers.

Keywords: Tolterodine tartrate; HPLC–ESI–MS; Human plasma; Pharmacokinetics

1. Introduction

Tolterodine tartrate, (*R*)-*N*,*N*-diisopropyl-3- (2-hydroxy-5-methylphenyl)-3-phenyl-propanamine L-hydrogen tartrate, is a new, potent and competitive muscarinic receptor antagonist in clinical development for the treatment of urge incontinence and other symptoms of unstable bladder. Tolterodine has a high affinity and specificity for muscarinic receptors in vitro and exhibits the selectivity for the urinary bladder over salivary glands in vivo, so it has the advantageous tolerability profile in terms of the low frequency of bothersome dry mouth. After oral administration, tolterodine is metabolized in liver, resulting in the formation of the 5-hydroxymethyl derivative, a major pharmacologically active metabolite. However, a small proportion of Caucasians showed a pharmacokinetic profile in accordance with poor metabolisers, having about ten times higher tolterodine concentrations but no measurable concentrations of the 5-hydroxymethyl derivative [1–3].

The therapeutic dose of tolterodine tartrate is only 4 mg every day given orally and the test tablet in this experiment is an extended-release formation, so the concentration of tolterodine tartrate in human plasma is very low [4], a sensitive analytical method is needed for its determination in plasma. Kumar et al. [5] developed an isocratic chiral HPLC method for the separation of tolterodine tartrate enantiomers but did not refer to determination in human plasma. Palmer et al. [6] established a GC–MS method to quantification of tolterodine, the limit of quantification (LOQ) in plasma of the method was only 0.5 ng/ml, which was insufficiently sensitive to enable full pharmacokinetics profiling of tolterodine tartrate. Swart et al. [7] developed a capillary column LC switching system coupled to electrospray ionization-tandem mass spectrometry for quantification of free drug concentrations of tolterodine and two metabolites in plasma, the LOQ was 0.05 ng/ml, but the analysis time was long; then they [8] improved their experience with the capillary SPE coupled

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directly to MS–MS method which reduced the analysis time. In this paper, we describe a more simple, selective and highly sensitive method by using high performance liquid chromatography coupled with electrospray ionization single quadrupole mass spectrometry for the determination of tolterodine tartrate in human plasma.

2. Experimental

2.1. Chemicals and reagents

Tolterodine tartrate extended release test tablets (batch no. 20031001, ER), tolterodine tartrate reference tablets (batch no. 20030901, IR) and tolterodine tartrate reference standard (99.9% purity) were supplied and identified by Nanjing M.R. Pharmaceutical Corporation (Nanjing, PR China); Oxybutynin reference standard (internal standard, 99.3% purity) was supplied and identified by Nanjing G.C. Pharmaceutical Corporation (Nanjing, PR China). Acetonitrile was HPLC grade and was purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

2.2. Instrumentation and operating conditions

2.2.1. Liquid chromatography

Liquid chromatography was performed using a Shimadzu LC-10AD HPLC system consisting of an autosampler (SIL-HTc). The column was a Shim-pack VP-ODS C_{18} (150 mm × 2.0 mm I.D.) and was operated at 40 °C. The mobile phase consisted of acetonitrile: water (10 mMCH_3COONH_4, pH 3.0) = 50:50 (v/v) was set at a flow rate of 0.2 ml/min.

2.2.2. Mass spectrometry

Mass spectrometric detection was performed using a Shimadzu LCMS-2010A quadrupole mass spectrometer with an electrospray ionization (ESI) interface. The ESI source was set at positive ionization mode. The [$(M + H)^+$, *m*/*z* 326.15] for tolterodine tartrate and [$(M + H)^+$, *m*/*z* 358.25] for oxybutynin were selected as detecting ions, respectively. The MS operating conditions were optimized as follows: nebulizer gas rate 1.5 l/min, CDL temperature 250 °C, block temperature 200 °C, probe voltage: +4.5 kV. The quantification was performed via peak-area. Data acquisition and processing were accomplished using Shimadzu LCMS solution Software for LCMS-2010A system.

2.3. Preparation of stock solutions

Stock solutions of tolterodine tartrate and oxybutynin (IS) were prepared in HPLC mobile phase at concentrations of 1.0 mg/ml and were stored at 4 °C.

Working solutions of tolterodine tartrate were prepared daily in HPLC mobile phase by appropriate dilution at 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 ng/ml and 1.0, 1.5 μg/ml.

The stock solution of oxybutynin was further diluted with HPLC mobile phase to prepare the working internal standard solution containing 100 ng/ml of oxybutynin.

2.4. Sample preparation and extraction procedure

A 1 ml aliquot of the collected plasma sample from a human volunteer was pipetted into a 10 ml centrifuge tube. The working internal standard solution (50 μl × 100 ng/ml) and 5 ml *n*-hexane: isopropanol (95:5, v/v) were added and then were vortexed for 2 min. After centrifugation of the sample at 1330 × *g* for 10 min, the organic layer was transferred to another 10 ml centrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas in a water bath at 40 °C. The residue was redissolved in 100 μl mobile phase. An aliquot of 10 μl was injected into the LC–MS system.

2.5. Standard curves

Proper volume of one of the above-mentioned working solutions to produce the standard curve points equivalent to 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0 and 30.0 ng/ml of tolterodine tartrate was added into 10 ml centrifuge tubes, respectively and evaporated to dryness under stream of nitrogen gas at 40 °C, and then redissolved in 1 ml blank plasma. Each sample also contained 5.0 ng of the internal standard. The following assay procedures were the same as describe above. In each run, a blank plasma sample (no IS) was also analyzed.

2.6. Preparation of quality control samples

Quality control samples were prepared daily by spiking different samples of 1 ml plasma each with proper volume of the corresponding standard solution to produce a final concentration equivalent to low level (0.20 ng/ml), middle level (10.0 ng/ml) and high level (30.0 ng/ml) of tolterodine tartrate with 5.0 ng/ml of internal standard each. The following procedures were the same as describe above.

2.7. Method validation

The method validation assays were carried out following the currently accepted US Food and Drug Administration (FDA) bioanalytical method validation guidance [9].

2.7.1. Assay specificity

Analyses of blank samples of the healthy human blank plasma were obtained from six sources. Each blank sample was tested for the visible interference.

In order to evaluate the matrix effect on the ionization of analytes, three different concentration levels of tolterodine tartrate (0.20, 10.0 and 30.0 ng/ml) were added to the dried

extracts of 1 ml blank sample respectively, then were dried and dissolved with 100 μl mobile phase. The same concentration levels of tolterodine tartrate were dried directly and dissolved with the same volume of the mobile phase. The matrix effect of internal standard (5.0 ng/ml) was evaluated using the same method.

2.7.2. Linearity

Standard curves of nine concentrations of tolterodine tartrate ranged 0.1–30.0 ng/ml were extracted and assayed. Blank plasma samples were analyzed to ensure the lack of interferences but not used to construct the calibration function. The lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio of 5 and 10, respectively.

2.7.3. Precision and accuracy

The precision of the assay was determined from the QC plasma samples by replicate analyses of three concentration levels of tolterodine tartrate (0.20, 10.0 and 30.0 ng/ml). Within-batch precision and accuracy was determined by repeated analysis of the group of standards on one day ($n = 5$). Between-batch precision and accuracy was determined by repeated analysis on three consecutive days ($n = 5$ series per day). The concentration of each sample was determined using standard curve prepared and analyzed on the same day.

2.7.4. Extraction recovery

The extraction recovery of tolterodine tartrate was determined by comparing the tolterodine tartrate/IS peak area ratios (R_1) obtained from extracted plasma samples with those (R_2) from standard solutions at the same concentration. This procedure was repeated for the three different concentrations of 0.20, 10.0 and 30.0 ng/ml.

2.7.5. Stability

2.7.5.1. Freeze and thaw stability. Three concentration levels of QC plasma samples were stored at the storage temperature (−20 °C) for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze-thaw cycle were repeated twice, then the samples were tested after three freeze (−20 °C)-thaw (room temperature) cycles.

2.7.5.2. Short-term temperature stability. Three concentration levels of QC plasma samples were kept at room temperature for a period that exceeded the routine preparation time of samples (around 6 h).

2.7.5.3. Long-term stability. Three concentration levels of QC plasma samples kept at low temperature (−20 °C) were studied for a period of 4 weeks.

2.7.5.4. Post-preparative stability. The autosampler stability was conducted reanalyzing extracted QC samples kept under the autosampler conditions (4 °C) for 12 h.

2.7.5.5. Stock solution stability. The stability of tolterodine tartrate and internal standard working solutions were evaluated at room temperature for 6 h.

2.7.6. Standard curve and quality control sample in each batch

A standard curve in each analytical run was used to calculate the concentration of tolterodine tartrate in the unknown samples in the run. It was prepared at the same time as the unknown samples in the same batch and analyzed in the middle of the run.

The QC samples in five duplicates at three concentrations (0.20, 10.0 and 30.0 ng/ml) were prepared and were analyzed with processed test samples at intervals per batch.

3. Clinical study design

Twenty healthy male subjects were enrolled in a randomized, two-treatment, two-period, single-dose and multiple-dose crossover study with a week washout between the first dosing in period I and the first dosing of period II. The design of drug administration is shown in Table 1.

3.1. Single dose study

Subjects fasted from the night before dosing until 2 h after dosing for each session. For tolterodine-IR group, the 4 mg tolterodine IR formulation was administered and blood samples were obtained prior to dose administration (time 0) and at 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0 and 24.0 h after the dose. For tolterodine-ER group, the 4 mg tolterodine ER formulation was administered and blood samples were obtained prior to dose administration (time 0) and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0 and 24.0 h after the dose. The blood samples were immediately centrifuged at 1600 × *g* for 10 min. The plasma was removed and stored at −20°C until analysis was done.

3.2. Multiple dose study

The 4 mg tolterodine ER formulation was administered to the subjects once daily for 6 days (at 0, 24, 48, 72, 96 and 120 h), and the 2 mg tolterodine IR formulation was administered every 12 h for 6 days (at 0, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h). Venous blood samples were obtained

Table 1
Design of drug administration

Treatment	Period I		Washout week	Period II	
	Day 1	Day 3–8	Day 9–14	Day 15	Day 17–22
I	S (T)	M (T)	Washout	S (R)	M (R)
II	S (R)	M (R)	Washout	S (T)	M (T)

S: single dose administration; M: multiple dose administration; T: test tablets; R: reference tablets.

at 16 time points from tolterodine-IR group (at 72, 96, 120, 120.33, 120.67, 121, 121.5, 122, 122.5, 123, 124, 126, 128, 132, 136 and 144 h) and at 16 time points from tolterodine-ER group (at 72, 96, 120, 120.5, 121, 121.5, 122, 123, 124, 125, 126, 128, 130, 132, 136 and 144 h). The blood samples were immediately centrifuged at 1600 × *g* for 10 min. The plasma was removed and stored at −20°C until analysis was done.

4. Results and discussion

4.1. Selection of IS

It is necessary to use an internal standard to get high accuracy when HPLC is equipped with MS as the detector. Oxybutynin was adopted in the end because of its similarity of retention action, ionization and extraction efficiency as well as less endogenous interference at *m/z* 358.25. The structures of the ionized (protonated) forms of tolterodine tartrate and oxybutynin are shown in Fig. 1.

4.2. Sample preparation

Liquid-liquid extraction was necessary and important because this technique can not only purify but also concentrate the sample. *n*-Hexane-isopropanol (95:5, v/v), ethyl acetate and methylene chloride-ethyl acetate (20:80, v/v) were all attempted and *n*-hexane-isopropanol (95:5, v/v) was finally adopted because of its high extraction efficiency and less interference. Extractions with and without adding 0.1 M NaOH (100 μl) were both tried, and obvious differences were not observed, so the extraction using *n*-hexane-isopropanol (95:5, v/v) without adding 0.1 M NaOH was used at last.

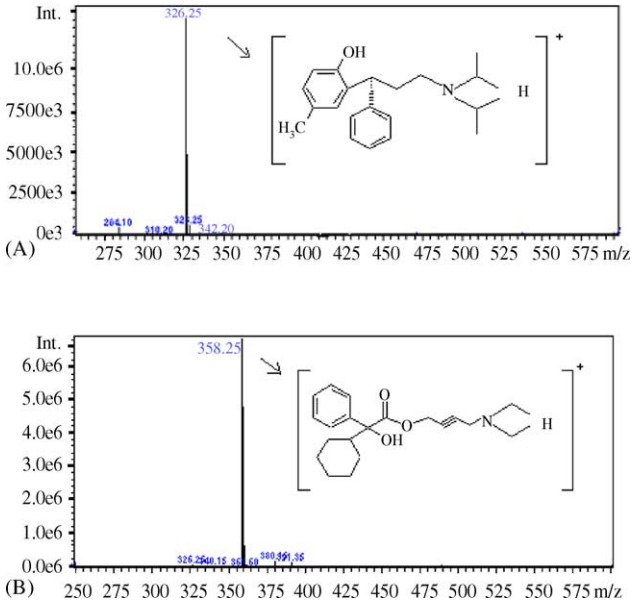

Fig. 1. Positive ion electrospray mass scan spectrum of: tolterodine tartrate (A) and oxybutynin (B).

4.3. Separation

Positive electrospray ionization mass scan spectra of tolterodine tartrate and IS were shown in Fig. 1, respectively. According to the mass scan spectrum, *m/z* 326.25 produced by the quasimolecule ion $[M + H]^+$ of tolterodine tartrate and *m/z* 358.25 produced by the quasimolecule ion $[M + H]^+$ of oxybutynin were selected for monitoring.

The selected-ion monitoring (SIM) (+) chromatograms extracted from supplemented plasma are depicted in Fig. 2C. As shown, the retention times of tolterodine tartrate and the IS were 2.4 and 3.2 min, respectively. They were well separated, which avoided the interference of ionization between them.

The total HPLC–MS analysis time was 5 min per sample. A representative chromatogram of a plasma sample obtained at 6 h from a subject who received a single oral dose (4 mg) is shown in Fig. 3.

4.4. Method validation

4.4.1. Assay specificity

No interferences of the analytes were observed. Fig. 2A shows an HPLC chromatogram for a blank plasma sample indicating no endogenous peaks at the retention positions of tolterodine tartrate or internal standard (oxybutynin). All the ratios of the peak area resolved in blank sample compared with that resolved in mobile phase are between 85 and 115%, which means no matrix effect for tolterodine tartrate and oxybutynin in this method.

4.4.2. Linearity and LLOQ

The quantification of tolterodine tartrate in plasma samples was carried out by determining the slope (*b*), intercept (*a*) and regression coefficient (*r*) of the standard curves of the peak area ratio of tolterodine tartrate/oxybutyinin versus tolterodine tartrate concentration. Using linear regression analysis, the data confirmed linear relationships over the selected concentration range. Standard curves were constructed on 5 different days. The mean standard curve was typically described by the least-square equation: $R = 0.2410 \times C + 0.0289$ ($r = 0.9993$), where R corresponds to the peak area ratio of tolterodine tartrate to the IS and C refers to the concentration of tolterodine tartrate added to plasma over a concentration range of 0.1–30.0 ng/ml. Results of five representative standard curves for tolterodine tartrate LC–MS determination are given in Table 2.

The lower limit of quantification for tolterodine tartrate was proved to be 0.10 ng/ml and the lower limit of detection was 0.05 ng/ml. Fig. 2B shows the chromatogram of an extracted sample that contained 0.10 ng/ml (LLOQ) of tolterodine tartrate.

4.4.3. Precision and accuracy

Data for within-batch and between-batch precision and accuracy of the method for tolterodine tartrate are presented

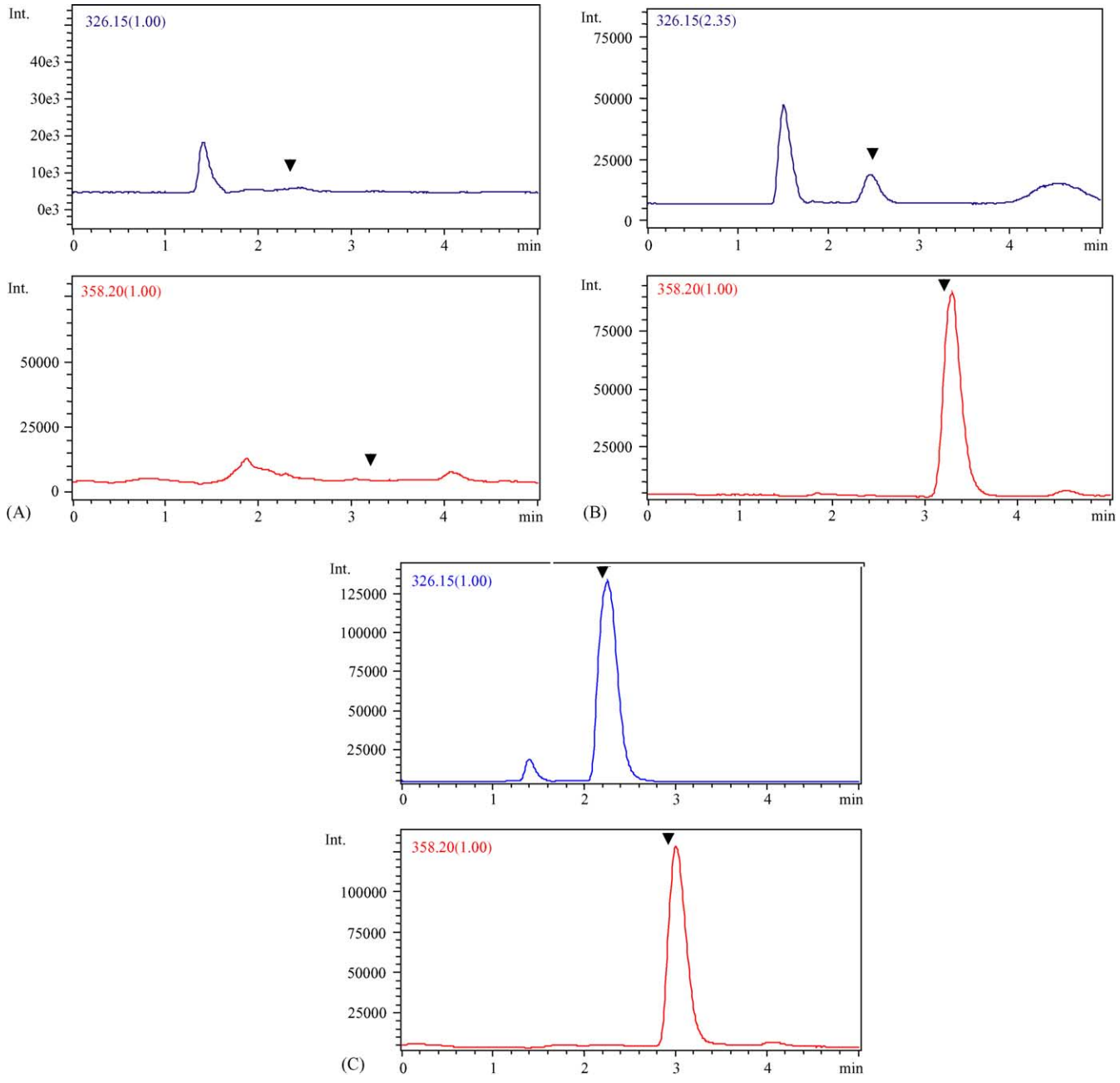

Fig. 2. The SIM (+) chromatograms of tolterodine tartrate and oxybutynin. Peaks were assigned with (▼).The retention times of tolterodine tartrate and the IS were 2.4 and 3.2 min, respectively. (A) Blank plasma. (B) LLOQ (concentration of tolterodine tartrate = 0.1 ng/ml). (C) Supplemented plasma (concentration of tolterodine tartrate = 5 ng/ml).

in Table 3. The accuracy deviation values are within 10% of the actual values. The precision determined at each concentration level does not exceed 10% of the relative standard deviation (R.S.D.). The results revealed good precision and accuracy.

4.4.4. *Extraction recovery*

The extraction recovery determined for tolterodine tartrate was shown to be consistent, precise and reproducible. Data was shown below in Table 4.

4.4.5. *Stability*

Table 5 summarizes the freeze and thaw stability, short-term stability, long-term stability and post-preparative stability data of tolterodine tartrate. All the results showed the stability behavior during these tests and there were no stability-related problems during the samples routine analysis for the pharmacokinetic, bioavailability or bioequivalence studies.

The stability of working solutions was tested at room temperature for 6 h. Based on the results obtained, these working solutions were stable within 6 h.

Table 2
Results of five representative standard curves for tolterodine tartrate LC–MS determination

Added concentration (ng/ml)	0.1	0.2	0.5	1.0	2.0	5.0	10.0	20.0	30.0
Back-calculated concentration	0.10	0.21	0.51	0.93	1.91	4.99	9.70	20.13	31.65
	0.10	0.21	0.50	1.03	1.98	5.34	9.25	18.85	30.49
	0.10	0.21	0.53	0.96	1.95	5.17	9.11	19.95	30.42
	0.11	0.21	0.51	1.03	1.91	5.34	10.17	20.70	31.37
	0.10	0.22	0.51	0.96	2.08	4.80	9.30	21.00	29.20
Mean	0.10	0.21	0.51	0.98	1.97	5.13	9.5	20.12	30.63
R.S.D. (%)	4.27	2.31	2.21	4.37	3.67	4.56	4.55	4.12	3.13
Accuracy (%)	98.40	106.30	102.44	98.36	98.28	102.54	95.05	100.62	102.08

Table 3
The within- and between-batch precision, accuracy of the method for determination of tolterodine tartrate (within-batch: $n = 5$; between-batch: $n = 5$ series per day)

Added concentration (ng/ml)	Within-batch			Between-batch		
	Detected concentration (mean ± S.D., ng/ml)	Mean accuracy (%)	R.S.D. (%)	Detected concentration (mean ± S.D., ng/ml)	Mean accuracy (%)	R.S.D. (%)
0.2	0.22 ± 0.01	107.58	4.61	0.20 ± 0.01	102.41	6.57
10.0	10.53 ± 0.41	105.28	3.94	10.56 ± 0.42	105.56	3.98
30.0	30.93 ± 0.96	103.11	3.09	30.66 ± 0.95	102.22	3.10

4.5. Results of pharmacokinetic study

4.5.1. Single dose study

Mean plasma concentration–time curve of tolterodine tartrate in 20 volunteers after oral administration of tolterodine tartrate in single dose study was showed in Fig. 4. Kinetic parameters were listed in Table 6. Significantly lower C_{max}

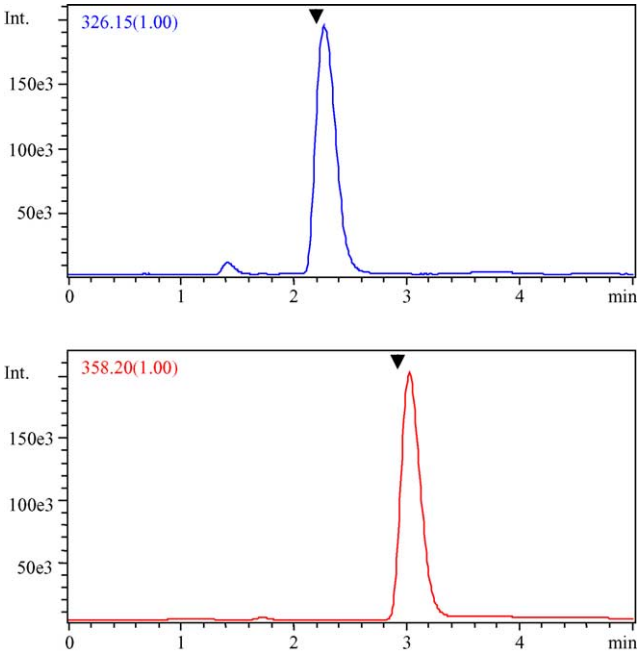

Fig. 3. The SIM (+) chromatograms for plasma sample of a healthy volunteer. Peaks were assigned with (▼). The retention times of tolterodine tartrate and the IS were 2.4 and 3.2 min, respectively. The concentration of tolterodine tartrate was 6.02 ng/ml.

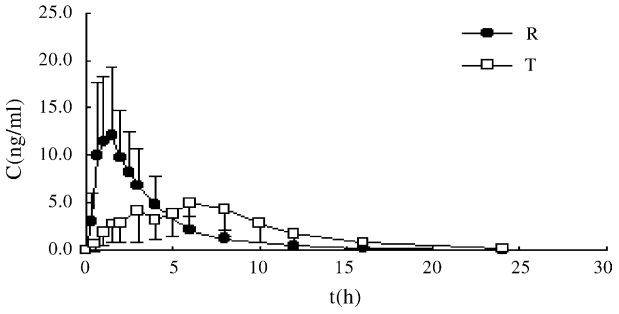

Fig. 4. Mean drug plasma concentration–time curve of tolterodine tartrate in 20 volunteers after oral administration of tolterodine tartrate in single dose study. R: reference tablets; T: test tablets.

Table 4
Recoveries of tolterodine tartrate from plasma ($n = 5$)

Added (ng/ml)	Recovery (mean ± S.D., %)	R.S.D. (%)
0.2	91.98 ± 8.98	9.76
10.0	90.84 ± 7.02	7.72
30.0	92.43 ± 4.25	4.60

Table 5
Data showing stability of tolterodine tartrate in human plasma at different QC levels ($n = 5$)

	Accuracy (mean ± S.D., %)		
	0.2 (ng/ml)	10.0 (ng/ml)	30.0 (ng/ml)
Short-term stability	90.14 ± 3.90	98.15 ± 4.14	97.26 ± 3.01
Freeze and thaw stability	92.53 ± 7.32	96.17 ± 3.46	94.11 ± 4.52
Long-term stability	90.43 ± 8.59	93.06 ± 4.53	91.25 ± 3.09
Post-preparative stability	92.28 ± 5.41	97.68 ± 2.27	96.37 ± 2.68

Table 6
Pharmacokinetic parameters of 20 healthy male volunteers after oral administration of tolterodine tartrate in single and multiple dose study

Parameters	Single dose study		Multiple dose study	
	Test	Reference	Test	Reference
$T_{1/2}$ (h)	3.01 ± 0.81	2.25 ± 0.77	–	
T_{max} (h)	5.4 ± 1.6	1.3 ± 0.5	5.1 ± 1.2	0.9 ± 0.3
C_{max} (ng ml^{-1})	6.08 ± 3.07	14.62 ± 7.53	6.72 ± 3.55	6.89 ± 3.44
AUC_{0-24} (ng h ml^{-1})	47.85 ± 28.40	45.94 ± 26.77	–	–
$AUC_{0-\infty}$ (ng h ml^{-1})	48.83 ± 29.37	46.46 ± 26.91	–	–
C_{min} (ng ml^{-1})	–	–	0.29 ± 0.31	0.06 ± 0.11
C_{av} (ng ml^{-1})	–	–	2.34 ± 1.36	1.22 ± 0.67
AUC_{ss} (ng h ml^{-1})	–	–	56.25 ± 32.69	29.20 ± 16.05
DF	–	–	2.89 ± 0.77	5.98 ± 2.01

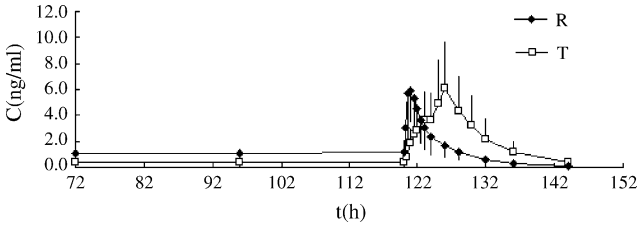

Fig. 5. Mean drug plasma concentration–time curve of tolterodine tartrate in 20 volunteers after oral administration of tolterodine tartrate in multiple dose study. R: reference tablets; T: test tablets.

and delayed T_{max} of tolterodine tartrate were observed with the ER formulations, which showed the extended release quality. The test extended release tablet was found to be bioequivalent to the reference one by comparing the AUC of ER and IR, and the mean relative bioavailability of ER was 105.0 ± 11.6%.

4.5.2. Multiple dose study

Mean plasma concentration–time curve of tolterodine tartrate in 20 volunteers after oral administration of tolterodine tartrate in multiple dose study were showed in Fig. 5. Kinetic parameters were listed in Table 6. The degree of fluctuation (DF) of ER was significantly less than those of IR and the mean relative bioavailability of ER was 94.7 ± 16.2%.

5. Conclusion

A sensitive, selective, accurate and precise HPLC method with selected ion monitoring by single quadrupole mass spectrometer with ESI interface was developed and validated for determination of tolterodine tartrate in human plasma. The reported method offers several advantages such as a rapid and simple extraction scheme, and a short chromatographic run time, which makes the method suitable for the analysis of large sample batches resulting from the pharmacokinetic, bioavailability or bioequivalent study of tolterodine tartrate.

The results of pharmacokinetic study showed that there were significant differences between individuals in the aspect of C_{max} and AUC, which suggested the individualization of drug dosage regimes.

References

[1] L. Nilvebrant, B. Hallen, G. Larsson, Life Sci. 60 (1997) 1129.
[2] E.S. Rovner, A.J. Wein, Eur. Urol. 41 (2002) 6.
[3] L. Nilvebrant, Life Sci. 68 (2001) 2549.
[4] B. Ollson, J. Szamosi, Clin. Pharmacokinet. 40 (2001) 227.
[5] Y.R. Kumar, G. Ralamu, V.V. Vevakanand, G. Vaidyanathan, K. Srinivas, M.K. Kumara, K. Mukkanti, M.S. Reddya, S. Venkatraman, M.V. Suryanarayana, J. Pharm. Biomed. Anal. 35 (2004) 1279.
[6] L. Palmer, L. Andersson, T. Andersson, U. Stenberg, J. Pharm. Biomed. Anal. 16 (1997) 155.
[7] R. Swart, P. Koivisto, K.E. Markides, J. Chromatogr. A 828 (1998) 209.
[8] R. Swart, P. Koivisto, K.E. Markides, J. Chromatogr. B 736 (1999) 247.
[9] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.