ORIGINAL ARTICLES

Institute of Pharmaceutical Science and Technology and College of Pharmacy[1], Hanyang University, Republic of Korea; College of Science & Engineering[2], Jungwon University, Republic of Korea

Plasma concentration profile of tolterodine and 5-hydroxymethyl tolterodine following transdermal administration of tolterodine in rats

Young Seok Ji [1,2], In Sook Kim[1], Tae Kon Kim[2,*], Hye Hyun Yoo[1,*]

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**Corresponding authors: Prof. T. K. Kim, College of Science & Engineering, Jungwon University, Geosan-gun, Chungbuk 367-700, Republic of Korea*
tkkim@jwu.ac.kr
Prof. H. H. Yoo, Institute of Pharmaceutical Science and Technology and College of Pharmacy, Hanyang University, Ansan, Gyeonggi-do 15588, Republic of Korea
yoohh@hanyang.ac.kr

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In this study, the plasma concentration profiles of tolterodine and its active metabolite, 5-hydroxymethyl tolterodine (5-HM tolterodine) were investigated in rats with tolterodine transdermal patches using liquid chromatography-tandem mass spectrometry. The plasma samples were extracted by a liquid-liquid extraction method, with an n-hexane/isopropyl alcohol mixture (9:1, v/v). Tiropramide was used as an internal standard (IS). Chromatographic separation was achieved using a C18 column (2.0 mm × 150 mm, 5 μm), with a mobile phase consisting of 5 mM ammonium acetate in distilled water/acetonitrile (50:50, v/v). The precursor-product ion pairs used for multiple reaction monitoring were m/z 326→284 (tolterodine), m/z 342→223 (5-HM tolterodine), and m/z 468→367 (IS). Subsequently, the plasma concentration levels of tolterodine and 5-HM tolterodine were measured in rat plasma after oral or transdermal administration of tolterodine and the pharmacokinetic parameters were calculated. The C_{max} of the patch was less than that of the oral administration but their AUC values were comparable. The resulting data suggested that a transdermal dose of tolterodine 3 times higher (9 mg/12 cm^2) could yield comparable efficacy to a 10 mg/kg oral dose in rats. These results would provide useful information on dose optimization of tolterodine transdermal patch for further clinical studies.

1. Introduction

Tolterodine is an antimuscarinic drug used for the treatment of urinary incontinence (Van Kerrebroeck et al. 1998). Tolterodine acts on all types of muscarinic receptors, but targets the bladder more than other organs or tissues and, thus, it has fewer side effects than other, older antimuscarinic drugs (Abrams et al. 1998; Cappon et al. 2008; Paquette et al. 2011).

Antimuscarinic agents inhibit muscarinic receptors in the urothelium and detrusor muscle and reduce the detrusor contraction and sensations associated with urgency (Andersson and Yoshida 2003). However, due to a wide distribution of muscarinic receptors in the body, antimuscarinic agents may cause a variety of adverse effects; dry mouth is the most commonly reported adverse effect (Abrams et al. 1998). Antimuscarinic agents are available mainly in oral tablets or capsules, but for the reason mentioned above, non-oral formulations such as transdermal patches have been considered. In addition, transdermal patch reduce pill burden, number of doses, and potential drug-drug interactions (Rajabalaya et al. 2017).

In an attempt to develop a tolterodine transdermal patch, we investigated the plasma pharmacokinetics of tolterodine and its major metabolite, 5-hydroxymethyl tolterodine (5-HM tolterodine) after transdermal administration of tolterodine in rats.

2. Investigations and results

2.1. Method validation

The representative liquid chromatography-tandem mass spectrometry (LC-MS/MS) extracted ion chromatograms for tolterodine, 5-HM tolterodine, and tiropramide (IS) in blank rat plasma (A), standard spiked plasma at the lower limit of quantitation (LLOQ) (B), and plasma obtained two hours after oral administration of tolterodine are shown in Fig. 1. The retention times of tolterodine, 5-HM tolterodine, and tiropramide were 2.5, 1.9, and 2.4 min, respectively, under the isocratic condition with 50:50 aqueous-organic mobile phase. The higher content of the organic solvent enabled more rapid elution of analytes and consequent shorter run time. However, the earlier elution of the analytes resulted in considerable ion suppression; thus the isocratic condition was optimized to minimize both matrix effects and elution time and the present condition was selected. Selectivity was evaluated using six different batches of blank plasma. No interfering peaks were observed at the retention times of tolterodine, 5-HM tolterodine, and tiropramide in the blank rat plasma.

Calibration curves showed a good linearity over the range 0.1–50 ng/mL for tolterodine and 5-HM tolterodine with a correlation determination (R^2) greater than 0.99. The LLOQ was 0.1 ng/mL for both analytes, satisfying the criteria of accuracy within 80 and 120 % and precision of ≤ 20%. In addition, the back-calculation data at the rest concentration points showed the accuracy and precision within ±15 % errors.

The intra- and inter-day assays for precision and accuracy are summarized in Table 1. As for tolterodine, the intra- and inter-day accuracy was between 97.0 and 118.6% with relative standard deviation less than 13.3%. As for 5-HM tolterodine, the intra- and inter-day accuracy was between 88.8% and 105.9% with relative standard deviation (RSD) less than 15.7%. The values exceeding 15% were all recorded at LLOQ levels. These results indicated that the present method satisfied the criteria for accuracy and precision.

The stability of tolterodine and 5-HM tolterodine in rat plasma was investigated under various storage and process conditions. Tolterodine and 5-HM tolterodine were stable at all tested concentrations with acceptable accuracy (89.0~104.3% and 91.2~106.7%, respectively) and precision (<10.5 and <8.2%, respectively), for 6 h at

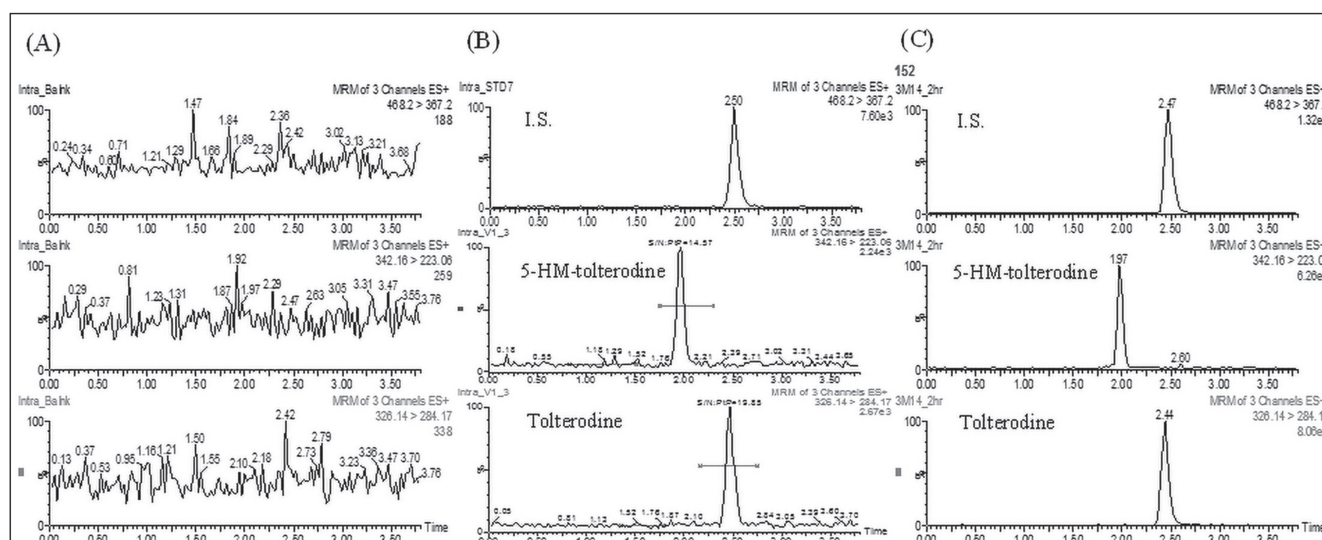

Fig. 1: Representative extracted ion chromatograms for tolterodine, 5-HM tolterodine, and IS for (A) blank rat plasma, (B) standard spiked plasma (at LLOQ: 0.1 ng/mL), and (C) plasma obtained 2 h from oral administration of tolterodine

Table 1: Intra- and inter-day accuracy and precision for tolterodine and 5-HM tolterodine in rat plasma

	Conc. (ng/mL)	Intra-day (*n*=5)		Inter-day (*n*=5)	
		Accuracy (%)	Precision (% RSD[a])	Accuracy (%)	Precision (% RSD)
Tolterodine	0.1	118.6	4.6	97.0	13.3
	0.3	105.7	4.2	100.4	3.4
	3	104.8	8.0	101.4	2.1
	40	105.2	1.3	104.2	3.4
5-HM tolterodine	0.1	100.2	6.5	95.6	15.7
	0.3	90.0	1.0	94.0	12.6
	3	88.8	5.8	99.7	5.6
	40	91.2	2.6	105.9	9.7

[a] % Relative standard deviation

room temperature, for four weeks at -70 °C, after three freeze-thaw cycles, and for 24 h in the autosampler. The working standard solutions of tolterodine, 5-HM tolterodine and IS were stable for 6 h at room temperature (accuracy 95.2~101.2% and precision < 5%). The mean extraction recoveries of tolterodine from rat plasma was 85.1% and 97.0% at concentrations of 0.3 and 30 ng/mL, respectively, with a maximum RSD of 9.2% (Table 2). The mean extraction recovery of 5-HM tolterodine from rat plasma was 95.5% and 96.9% at concentrations of 0.3 and 30 ng/mL, respectively, with a maximum RSD of 7.0% (Table 2). These data show that tolterodine and 5-HM tolterodine were efficiently extracted from plasma by the extraction procedure used.

The matrix effect was evaluated using a post-extraction addition approach. The mean recoveries of tolterodine were 81.6 and 99.6%, respectively, at concentrations of 0.3 and 3 ng/mL, respectively, and the variabilities between different sources of plasma samples (n=6) were 7.2 and 5.3%, as a relative standard deviation (%)(Table 2). The mean recoveries of 5-HM tolterodine were 83.0 and 98.4% at 0.3 and 3 ng/mL, and the RSD (n=6) were 1.9 and 7.7% (Table 2). The absolute matrix effects were somewhat greater at a lower concentration but the relative matrix effects were satisfactory at both concentrations.

Table 2: Recovery and matrix effect of tolterodine and 5-HM tolterodine in rat plasma

	Conc. (ng/mL)	Recovery (*n*=3)		Matrix effect (*n*=6)	
		Mean (%)	RSD[a] (%)	Mean (%)	RSD[a] (%)
Tolterodine	0.3	85.1	9.2	81.6	7.2
	3	97.0	1.5	99.6	5.3
5-HM tolterodine	0.3	95.5	7.0	83.0	1.9
	3	96.9	1.2	98.4	7.7

[a] % Relative standard deviation

As the linearity range investigated in this study is somewhat narrow, dilution integrity was evaluated for the application to samples at higher concentration levels. For the evaluation of dilution integrity, spiked plasma samples were prepared at 200 and 400 ng/mL for both analytes and diluted to 40 ng/mL with blank rat plasma and analyzed, and the concentrations of the spiked plasma samples using dilution factors. The calculated concentrations and precisions are presented in Table 3. All calculated values met the validation criteria, which indicated that dilution with the matrix did not affect the final results and higher concentration samples can be analyzed using this method.

Table 3: Evaluation of dilution integrity for tolterodine and 5-HM tolterodine (n=3)

Concentration (ng/mL)	Dilution to (ng/mL)	Tolterodine			5-HM tolterodine		
		Measured conc. (ng/mL)	Accuracy (%)	RSD[a] (%)	Measured conc. (ng/mL)	Accuracy (%)	RSD (%)
200	40	37.3 ± 0.6	93.2	1.5	34.9 ± 0.4	87.2	1.3
400		36.5 ± 1.5	91.2	4.2	36.1 ± 0.4	90.3	1.1

[a] % Relative standard deviation

2.2. *Pharmacokinetic study with tolterodine patches*

The developed method was applied to a pharmacokinetic study for tolterodine and 5-HM tolterodine after oral and transdermal (i.e., patch) administration of tolterodine to rats. The plasma concentration-time profiles of tolterodine and 5-HM tolterodine are presented in Fig. 2. After oral administration, tolterodine was detected in plasma until 24 h and 5-HM tolterodine until four hours. In the case of the patch, tolterodine was detected until 84 h, but 5-HM tolterodine was not detected in any samples. The resulting pharmacokinetic parameters are listed in Table 4. After oral administration of tolterodine at a dose of 10 mg/kg, the C_{max} values of tolterodine and 5-HM tolterodine were 3.00±0.54 ng/mL and 0.27±0.06 ng/mL, respectively, and the AUC_{last} values were 20.4±10.5 ng·h/mL and 0.82±0.34 ng·h/mL, respectively. As for the patch (tolterodine 3 mg/4cm^2), the C_{max} value of tolterodine was 0.55±0.37 ng/mL and the AUC_{last} value was 24.8±16.8 ng·h/mL.

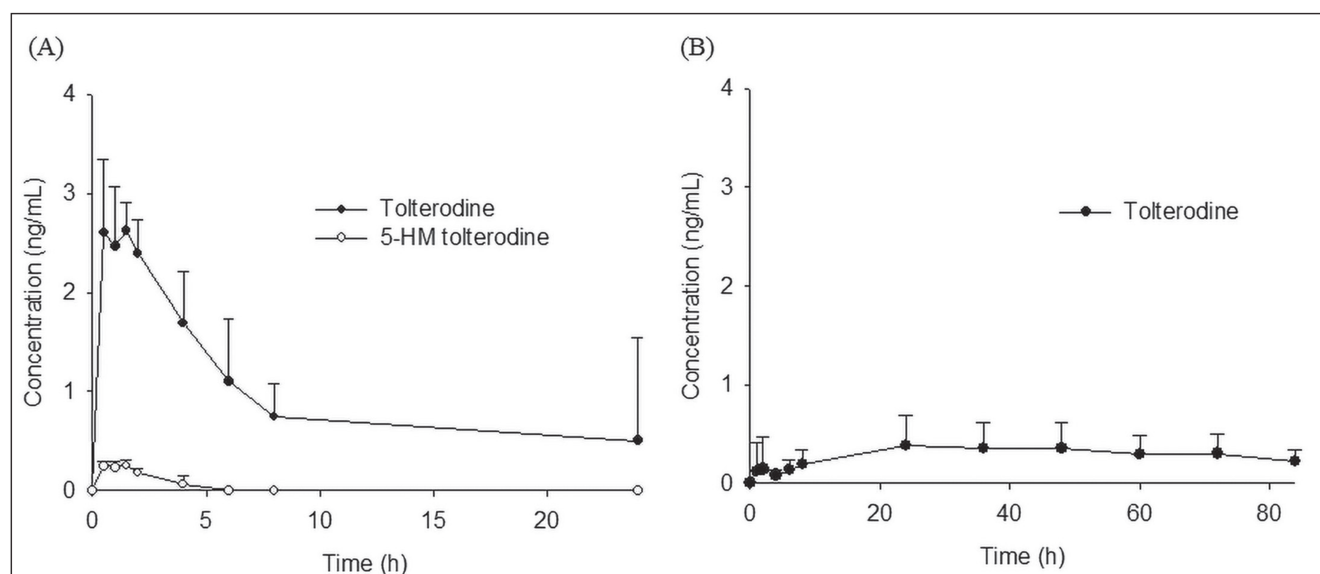

Fig. 2: Mean plasma concentration-time plots for tolterodine and 5-HM tolterodine in (A) oral (10 mg/kg, *n*=5) and (B) transdermal (3 mg/4cm^2 as a patch, *n*=10) administration. Data are presented as mean + standard error.

Table 4: Pharmacokinetic parameters for tolterodine and 5-HM tolterodine in rats

	Oral (10 mg/kg, *n*=5)		Patch (3 mg/4 cm^2, *n*=10)
	Tolterodine	5-HM-tolterodine	Tolterodine
AUC (ng·h/mL)	20.4 ± 10.5	0.82 ± 0.34	24.8 ± 16.8
C_{max} (ng/mL)	3.00 ± 0.54	0.27 ± 0.06	0.55 ± 0.37
T_{max} (h)	1.3 ± 0.5	1.1 ± 0.4	20.3 ± 12.5

3. Discussion

We investigated the plasma pharmacokinetics of tolterodine and its major metabolite, 5-HM tolterodine after oral and transdermal administration of tolterodine in rats. Tolterodine is metabolized by the cytochrome P450 2D6 (CYP2D6) enzyme to yield a pharmacologically active metabolite, 5-HM tolterodine. CYP2D6 shows phenotypical variability, due to genetic polymorphism. Therefore, for extensive metabolizers that have normal CYP2D6 function, the therapeutic effect of tolterodine is related to the sum of the plasma concentrations of tolterodine and 5-HM tolterodine. In this context, the plasma levels of both tolterodine and 5-HM tolterodine should be monitored to characterize the pharmacokinetic properties of this drug (Oishi et al. 2010).

The pharmacokinetics of tolterodine and 5-HM tolterodine have been undergoing steady investigation, due to their pharmacogenomic issues and for their application to new pharmaceutical formulations (Idkaidek et al. 2016; Liu et al. 2017; Oishi et al. 2011; Sun et al. 2013). Generally, the pharmacokinetic profiles of tolterodine and 5-HM tolterodine are highly variable in individual subjects due to genetic differences. The bioavailability of tolterodine is reported to be quite variable, ranging from 10 to 70% and the elimination half-life is reported to be 2~3 h in humans (Brynne et al. 1997).

In the present study, the pharmacokinetic data showed that the C_{max} of the patch was less than that of the oral administration but their AUC values were comparable. The concentration levels of 5-HM tolterodine were approximately 10 times less than that of tolterodine and the AUC was 20 times less than that of tolterodine in oral administration. Accordingly, after transdermal administration, 5-HM tolterodine may not be detected due to its probably low concentration levels as well as exemption from first pass metabolism. The mean plasma concentration for 24 h of the oral and transdermal formulations were calculated to be 0.845 ng/mL and 0.303 ng/mL, respectively. Therefore, a dose of tolterodine 3 times higher (9 mg/12 cm^2) could yield comparable efficacy to a 10 mg/kg oral dose in rats in terms of the mean plasma concentration considering the effective therapeutic window. In addition, the resulting pharmacokinetic profiles suggest that the patch formulation can reduce the number of doses as it shows pharmacokinetic pattern with a longer half-life. For example, bi-weekly (two times a week) or weekly patches can be developed. These results would provide useful information on dose optimization of tolterodine transdermal patch for further clinical studies.

4. Experimental

4.1. Chemicals

Tolterodine tartrate and 5-HM tolterodine were provided by SK Chemicals Ltd. (Seongnam, Korea), with a chemical purity of more than 98%. Tiropramide hydrochloride, used as an internal standard (IS), was provided by Hwail Pharma. Ltd. (Hwasung, Korea), with a chemical purity of 98%. The tolterodin patch was provided by SK Chemicals Ltd.. The patch was manufactured as a three-layered matrix patch consisting of backing film, acrylic adhesive matrix containing tolterodine, and release liner. Formic acid and ammonium acetate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Distilled water was prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade and were used as received. Blank plasma was collected from male Sprague-Dawley rats using heparin tubes.

4.2. Animal experiments

Male Sprague-Dawley rats (seven weeks old, weighing approximately 220–230 g) were purchased from Koatec Co. Ltd. (Pyeongtaek, Korea). The rats (n = 10) were housed in a temperature- (23±3 °C) and moisture- (55±15%) controlled room, exposed to a controlled 12-hour light/dark cycle, and allowed access to food and water *ad libitum*. The rats were divided into two groups—oral (group A) and patch-administered (group B) rats—by a stratified randomization procedure based on body weight. The carotid artery was cannulated with a polyethylene tube (PE-50), under ether anesthesia, one day before the administration. The rats were fasted overnight before and for six hours after drug administration, housed on wire bottom cages. For group A, the rats were orally administered a dose of 10 mg/kg tolterodine with a catheter. For group B, the rats were transdermally administered a dose of 3 mg tolterodine, using a 4 cm^2 patch, after shaving the villus of the rat abdomen with a razor. After administration, blood (250 µL) was collected in a heparinized tube at designated time points. For group A, blood was collected at 0.5, 1.5, 1, 2, 4, 6, 8, and 24 h after administration. For group B, blood was collected at 0, 1, 2, 4, 6, 8, 24, 36, 48, 60, 72, and 84 h after administration. Plasma was harvested after centrifugation at 3,000 rpm and 4 °C for 10 min and stored, frozen, at -70 °C until it was analyzed. The animal experimental protocol was approved by the Experimental Animal Care and Use Committee of SK Chemicals Ltd., and all animal experiments were performed in accordance with the Animal Experimentation Policy of SK Chemicals Ltd.

4.3. Sample preparation

A 10 µL aliquot of the IS solution (100 ng/mL tiropramide in methanol) and a 10 µL aliquot of 1 M NaOH were added to 100 µL of plasma. n-Hexane/isopropyl alcohol mixture (9:1, v/v; 600 µL) was added to the plasma sample, shaken for 30 min, and centrifuged at 3,000 × g for 5 min. The supernatant was taken and dried under an

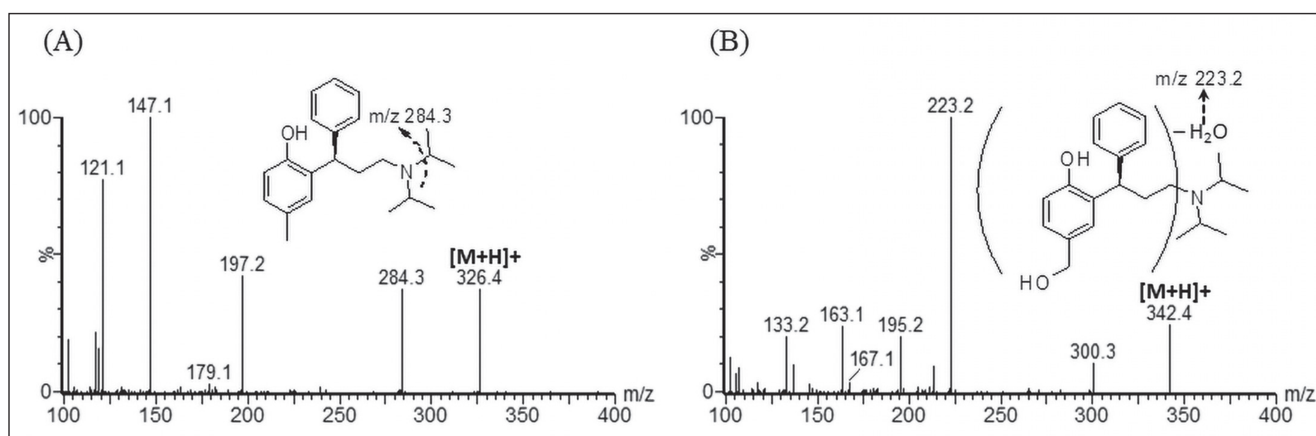

Fig. 3: Product mass spectra of tolterodine and 5-HM tolterodine

N_2 stream at 45 °C. The residue was reconstituted with 100 µL of the mobile phase solvent and transferred into a vial. A 20 µL aliquot was injected into the LC-MS/MS system.

4.4. Calibration standards and quality control samples

The stock standard solution of tolterodine and 5-hydroxymethyl tolterodine was prepared at a concentration of 1 mg/mL, in 50% acetonitrile in distilled water. The working standard solutions were prepared by serial dilution of the stock standard solution with 50% acetonitrile in distilled water. For preparation of calibration standards, 10 µL aliquots of the working standard solution were added to 90 µL of blank plasma to yield the final concentrations of 0.1, 0.2, 0.5, 1, 5, 10, and 50 ng/mL, for both analytes. Quality control (QC) samples were prepared at four concentration levels (0.1, 0.3, 3, and 40 ng/mL) in the same way. The samples were kept at -70 °C until analysis.

4.5. LC-MS/MS analysis

All analyses were performed using a Quattro Premier XE system equipped with a Waters 2795 Alliance HT HPLC system (Waters, Milford, MA, USA). Chromatographic separation was performed using a Gemini C18 column (2.0 mm × 150 mm, 5 µm, Torrance, CA, USA). The HPLC mobile phase consisted of a mixture of 5 mM ammonium acetate in distilled water/acetonitrile/formic acid (50:50:0.1, v/v) for isocratic elution. The flow rate was 0.2 mL/min. The total run time was 4 min. Mass spectrometric detection was operated in positive ion mode. The precursor-product ion pairs used for multiple reaction monitoring were m/z 326→284 for tolterodine, 342→223 for 5-HM tolterodine, and m/z 468→367 for IS (tiropramide). The product ion spectra for tolterodine and 5-HM tolterodine are shown in Fig. 3.

4.6. Method validation

The analytical method was validated in terms of selectivity, matrix effect, carry-over, linearity, precision, accuracy, and stability according to the "Guidance for Industry, Bioanalytical Method Validation" presented by the U.S. Food and Drug Administration (Guidance for Industry. 2013). Briefly, selectivity was evaluated by comparing chromatograms between blank and standard-spiked plasma samples with six different batches of blank plasma. Linearity was evaluated based on the correlation determination (R^2) of the calibration curve for peak-area ratios of analyte to IS *versus* the analyte concentrations. The LLOQ was defined as the lowest concentration with an accuracy of 80–120% and a RSD of <20%. Accuracy and precision were evaluated by repeated analyses of QC samples on five consecutive days. Extraction recovery was tested by comparing the peak area in the pre-spiked sample, with that in the post-spiked sample (n=3). Matrix effects were evaluated by comparing the peak area in the post-spiked (after extraction) sample with that in a neat solution using six different batches of blank plasma. For stability test, freeze and thaw stability (3 cycle, -70 °C), bench-top stability (6 h, room temperature), long-term stability (4 weeks, -70 °C), working standard solution stability (6 h, room temperature), and processed sample stability (24 h, 4 °C in autosapler) were evaluated (n=3). Recovery, matrix effect and stabilities were tested at concentrations of 0.3 and 3 ng/mL.

4.7. Pharmacokinetic analysis

The total area under the plasma concentration-time curve to the last time (AUC_{last}), maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) were calculated using WinNonlin™ 5.2 (Pharsight, Sunnyvale, CA, USA).

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Conflicts of interest: None declared.

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