



ORIGINAL ARTICLE

A validated LC–MS/MS method for the determination of tolterodine and its metabolite in rat plasma and application to pharmacokinetic study

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Abstract Liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was used for simultaneous quantification of tolterodine and its metabolite 5-hydroxy methyl tolterodine in rat plasma. Tolterodine-d6 and 5-hydroxy methyl tolterodine-d14 were used as internal standards (IS). Chromatographic separation was performed on Ascentis Express RP amide (50 mm × 4.6 mm, 2.7 μm) column with an isocratic mobile phase composed of 10 mM ammonium acetate and acetonitrile in the ratio of 20:80 (v/v), at a flow-rate of 0.5 mL/min. Tolterodine, tolterodine-d6, 5-hydroxy methyl tolterodine and 5-hydroxy methyl tolterodine-d14 were detected with proton adducts at *m/z* 326.1 → 147.1, 332.3 → 153.1, 342.2 → 223.1 and 356.2 → 223.1 in multiple reaction monitoring (MRM) positive mode respectively. The drug, metabolite and internal standards were extracted by liquid–liquid extraction method. The method was validated over a linear concentration range of 20.00–5000.00 pg/mL for tolterodine and 20.00–5000.00 pg/mL for 5-hydroxy methyl tolterodine. This method demonstrated intra- and inter-day precision of 0.62–6.36% and 1.73–4.84% for tolterodine, 1.38–4.22% and 1.62–4.25% for 5-hydroxy methyl tolterodine respectively. This method also demonstrated intra- and inter-day accuracy of 98.75–103.56% and 99.20–104.40% for tolterodine, 98.08–104.67% and 98.73–103.06% for

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5-hydroxy methyl tolterodine respectively. Both analytes were found to be stable throughout freeze–thaw cycles, bench top and postoperative stability studies. This method was successfully applied for the pharmacokinetic analysis of rat plasma samples following i.v. administration.

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1. Introduction

Tolterodine tartarate is chemically (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen tartarate. The molecular formula is $C_{26}H_{37}NO_7$ with a molecular weight of 473.58. Tolterodine is a competitive muscarinic receptor antagonist. Both urinary bladder contraction and salivation are mediated via cholinergic muscarinic receptors. After oral administration, tolterodine is metabolized in the liver, resulting in the formation of the 5-hydroxymethyl derivative, a major pharmacologically active metabolite. The 5-hydroxymethyl metabolite, which exhibits an antimuscarinic activity similar to that of tolterodine, contributes significantly to the therapeutic effect. Both tolterodine and the 5-hydroxymethyl metabolite exhibit a high specificity for muscarinic receptors, since both show negligible activity and affinity for other neurotransmitter receptors and other potential cellular targets, such as calcium channels. Tolterodine has a pronounced effect on bladder function. The main effects of tolterodine are an increase in residual urine, reflecting an incomplete emptying of the bladder, and a decrease in detrusor pressure, consistent with an antimuscarinic action on the lower urinary tract.

Both tolterodine and its active metabolite, 5-hydroxy methyl tolterodine, act as competitive antagonists at muscarinic receptors. This results in the inhibition of bladder contraction, a decrease in detrusor pressure, and an incomplete emptying of the bladder. In a study with ^{14}C -tolterodine solution in healthy volunteers who received a 5-mg oral dose, at least 77% of the radiolabeled dose was absorbed. C_{max} and area under the concentration–time curve (AUC) determined after dosage of tolterodine immediate release are dose-proportional over the range of 1–4 mg. C_{max} and C_{min} levels of tolterodine extended release are about 75% and 150% of tolterodine immediate release, respectively. Maximum serum concentrations of tolterodine extended release are observed 2–6 h after dose administration [1–9].

Literature survey reveals that few analytical methods were reported for quantification of tolterodine by using capillary electrophoresis [10], HPLC [11–14], UPLC [15], GC–MS [16], LC–MS [17–22]. Among all, quantification was done in pharmaceutical compounds [10–14] and biological samples [15–22]. Bioanalysis is most predominant in clinical pharmacokinetic studies. The bioanalytical methods by using LC–MS in human plasma [15–20] and human urine [15,16,21] were reported. No method was reported for quantification of tolterodine in rat plasma by using LC–MS/MS. However, only one method was reported where tolterodine was used as an internal standard [22]. LC–MS/MS has become a powerful technique in bioanalytical aspect to estimate pharmacokinetic parameters [23–25]. The aim of the present study is to develop and validate a simple, sensitive, rugged, reproducible, and most economical method for the quantification of tolterodine in rat plasma, which could be applied to the pharmacokinetic study.

2. Materials and methods

2.1. Chemicals

Tolterodine, 5-hydroxy methyl tolterodine, tolterodine-d6 and 5-hydroxy methyl tolterodine-d14 (Fig. 1) were obtained from Aurobindo Pharma Ltd. (Hyderabad, India). Ammonium acetate (reagent grade) was obtained from sd-fine chemicals (Mumbai, India). Methanol, acetonitrile and methyl-t-butyl ether (HPLC grade) were obtained from J.T. Baker (Mumbai, India).

2.2. LC–MS/MS instrument

The 1200 Series HPLC system (Agilent Technologies, Waldbronn, Germany) was used. Mass spectrometric detection was performed

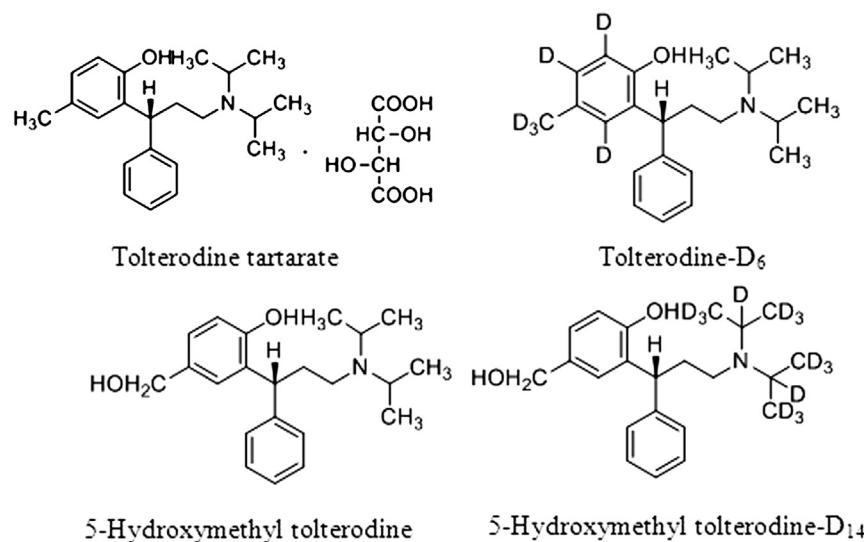


Fig. 1 Chemical structures of tolterodine, tolterodine-d6, 5-hydroxy methyl tolterodine, and 5-hydroxy methyl tolterodine-d14.

on an API 4200 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada). Data processing was performed on Analyst 1.5.1 software package (SCIEX).

2.3. Detection

In the Turbo ion spray positive mode with Unit Resolution, multiple reaction monitoring (MRM) was used for the detection. For tolterodine and 5-hydroxy methyl tolterodine the $[M-H]^+$ ions were monitored at m/z 326.1 and m/z 342.2 as the precursor ion, and a fragment at m/z 147.1 and m/z 223.1 as the product ion, respectively. For internal standard tolterodine-d6, 5-hydroxy methyl tolterodine-d14 the $[M-H]^+$ ions were monitored at m/z 332.3, m/z 356.2 as the precursor ion and a fragment at m/z 153.1 and m/z 223.1 as the product ion, respectively. Mass parameters were optimized as source temperature 550 °C, nebulizer gas 20 (nitrogen) psi, heater gas 30 (nitrogen) psi, curtain gas 20 (nitrogen) psi, CAD gas 5 (nitrogen) psi, ion spray (IS) voltage 5000 V, source flow rate 500 μ L/min without split, entrance potential 10 V, collision cell exit potential (CXP) 12 V, declustering potential (DP) 50 V, collision energy 35 V for tolterodine, tolterodine-d6, 5-hydroxy methyl tolterodine and 5-hydroxy methyl tolterodine-d14.

2.4. Chromatographic conditions

Ascentis Express RP amide (50 mm \times 4.6 mm, 2.7 μ m) was selected as the analytical column. The mobile phase was composed of 10 mM ammonium acetate and acetonitrile in the ratio of 20:80 (v/v). The flow rate of the mobile phase was set at 0.5 mL/min. The column temperature was set at 20 °C. Tolterodine-d6 and 5-hydroxy methyl tolterodine-d14 were found to be appropriate internal standards in terms of chromatography and extractability. The retention times of tolterodine, tolterodine-d6, 5-hydroxy methyl tolterodine, and 5-hydroxy methyl tolterodine-d14 were found to be approximately 1.9 ± 0.2 min, 1.9 ± 0.2 min, 1.2 ± 0.2 min and 1.2 ± 0.2 min, respectively.

2.5. Preparation of calibration standards and quality control (QC) samples

Standard stock solutions of tolterodine, 5-hydroxy methyl tolterodine (50.00 μ g/mL), and tolterodine-d6, 5-hydroxy methyl tolterodine-d14 (50.00 μ g/mL) were prepared in methanol. The spiking solutions for tolterodine-d6 (6000.00 pg/mL) and 5-hydroxy methyl tolterodine-d14 (5000.00 pg/mL) were prepared in 60% methanol from respective standard stocks. Standard stock solutions and IS spiking solutions were stored in a refrigerator at 2–8 °C until analysis. Standard stock solutions were added to drug-free rat plasma to obtain tolterodine/5-hydroxy methyl tolterodine concentration levels of 20.0, 40.0, 100.0, 250.0, 500.0, 1000.0, 2000.0, 3000.0, 4000.0/4000.0, and 5000.0 pg/mL for analytical standards and 20.0, 60.0, 1500.0, 3500.0, 3500.00 pg/mL for quality control standards. These standard solutions were stored at –30 °C in a freezer until analysis. The aqueous standards were prepared in reconstitution solution (10 mM ammonium acetate and acetonitrile in the ratio of 20:80 (v/v)), for validation exercises until analysis.

2.6. Sample preparation

Liquid–liquid extraction was used to isolate tolterodine/5-hydroxy methyl tolterodine and its respective IS from rat plasma. 50 μ L of IS (6000.00/5000.00 pg/mL) and 100 μ L of plasma sample (respective concentration) were added into labeled polypropylene tubes or ria vials, placed in an icebath and vortexed briefly. Then 2.5 mL of extraction solvent (methyl t-butyl ether) was added, closed with tight caps and vortexed for 10 min followed by centrifugation at 4000 rpm for 10 min at 20 °C. Then the supernatant from each sample was transferred into labeled polypropylene tubes. After that, the samples were evaporated to dryness at 40 °C under nitrogen. The dried residue samples were reconstituted with 100 μ L of reconstitution solution (acetonitrile:10 mM ammonium acetate:80:20, v/v) and vortexed briefly. Finally, sample from each tube was transferred into autosampler vials for injection.

2.7. Method validation

2.7.1. Linearity, precision and accuracy

The analytical curves were constructed using concentrations ranging from 20.00 to 5000.00 pg/mL for tolterodine and 20.00 to 5000.00 pg/mL for 5-hydroxy methyl tolterodine in rat plasma. Calibration curves were obtained by weighted 1 linear regression analysis. The peak area ratio of tolterodine to tolterodine-d6 was plotted against the tolterodine concentration in pg/mL. Similarly, the peak area ratio of 5-hydroxy methyl tolterodine to 5-hydroxy methyl tolterodine-d14 was plotted against the 5-hydroxy methyl tolterodine concentration in pg/mL. The correlation coefficients >0.9998, 0.9987 were obtained for both tolterodine and 5-hydroxy methyl tolterodine respectively. Precision and accuracy for the back calculated concentrations of the calibration points should be within $\leq 15\%$ and $\pm 15\%$ of their nominal values [26]. However, for LLOQ the precision and accuracy should be within $\leq 20\%$ and $\pm 20\%$ of their nominal values.

2.7.2. Recovery

The extraction recovery of tolterodine/5-hydroxy methyl tolterodine and tolterodine-d6/5-hydroxy methyl tolterodine-d14 from rat plasma was determined by analyzing quality control samples. Recovery at three concentrations (60.00, 1500.00 and 3500.00 pg/mL) was determined by comparing peak areas obtained from the plasma sample and those from the standard solution spiked with the blank plasma residue. A recovery of more than 50% was considered adequate to obtain the required sensitivity.

2.7.3. Limits of quantification (LOQ) and detection (LOD)

The response (peak area) was determined in blank plasma samples (six replicates from different plasma samples) and spiked LOQ sample prepared from the same plasma. The peak area of blank samples should not be more than 20% of the mean peak area of LOQ of tolterodine/5-hydroxy methyl tolterodine and not more than 5% of tolterodine-d6/5-hydroxy methyl tolterodine-d14. The precision and mean accuracy of the back calculated LOQ replicate concentrations must be ≤ 20 and $\pm 20\%$, respectively.

LOD is a parameter that provides the lowest concentration in a sample that can be detected from background noise but not quantitated. LOD was determined using the signal-to-noise ratio (S/N) of 3:1 by comparing test results from samples with known concentrations of analyte with blank samples.

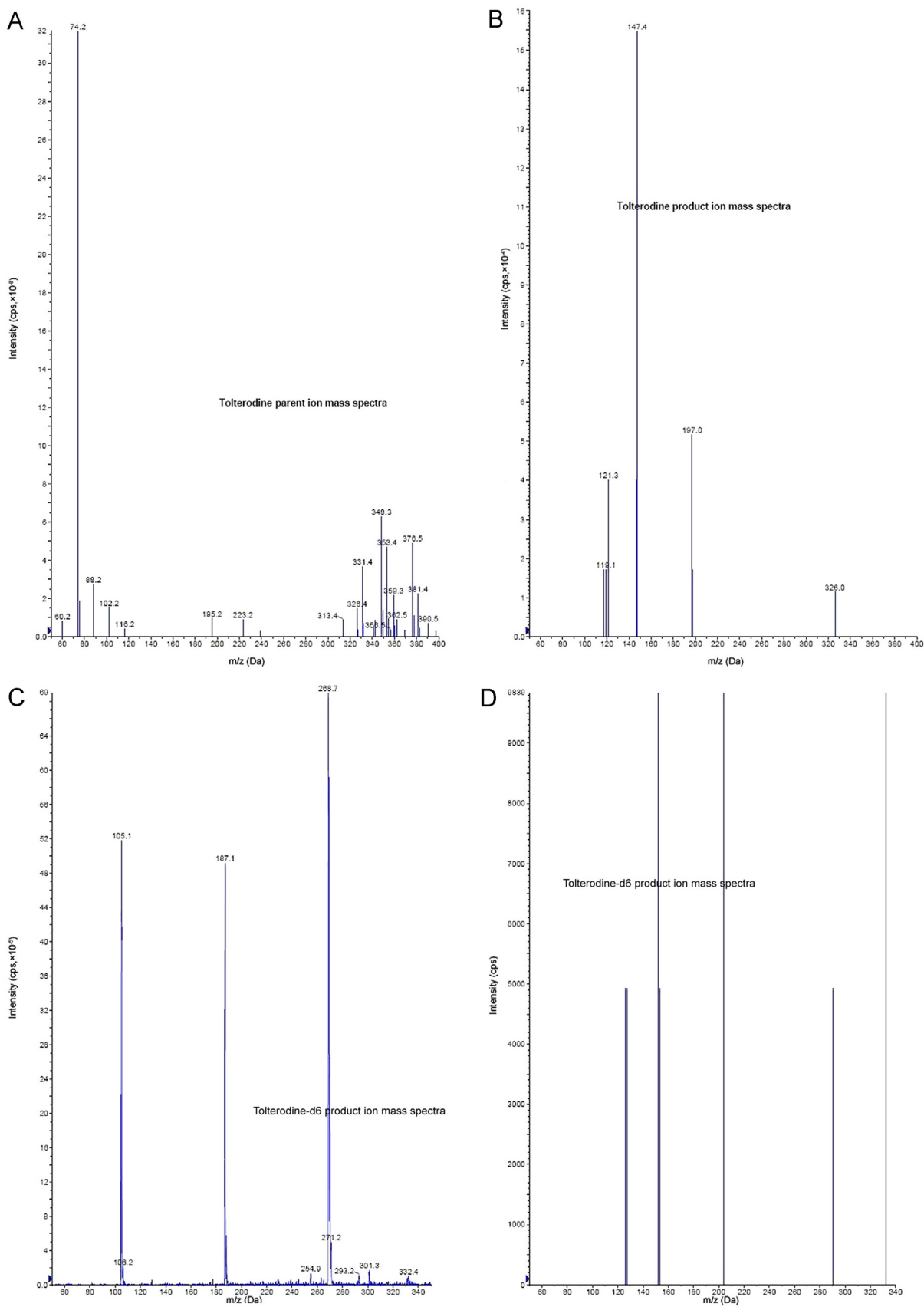


Fig. 2 Mass spectra of (A) tolterodine parent ion, (B) tolterodine product ion, (C) tolterodine-d6 parent ion and (D) tolterodine-d6 product ion.

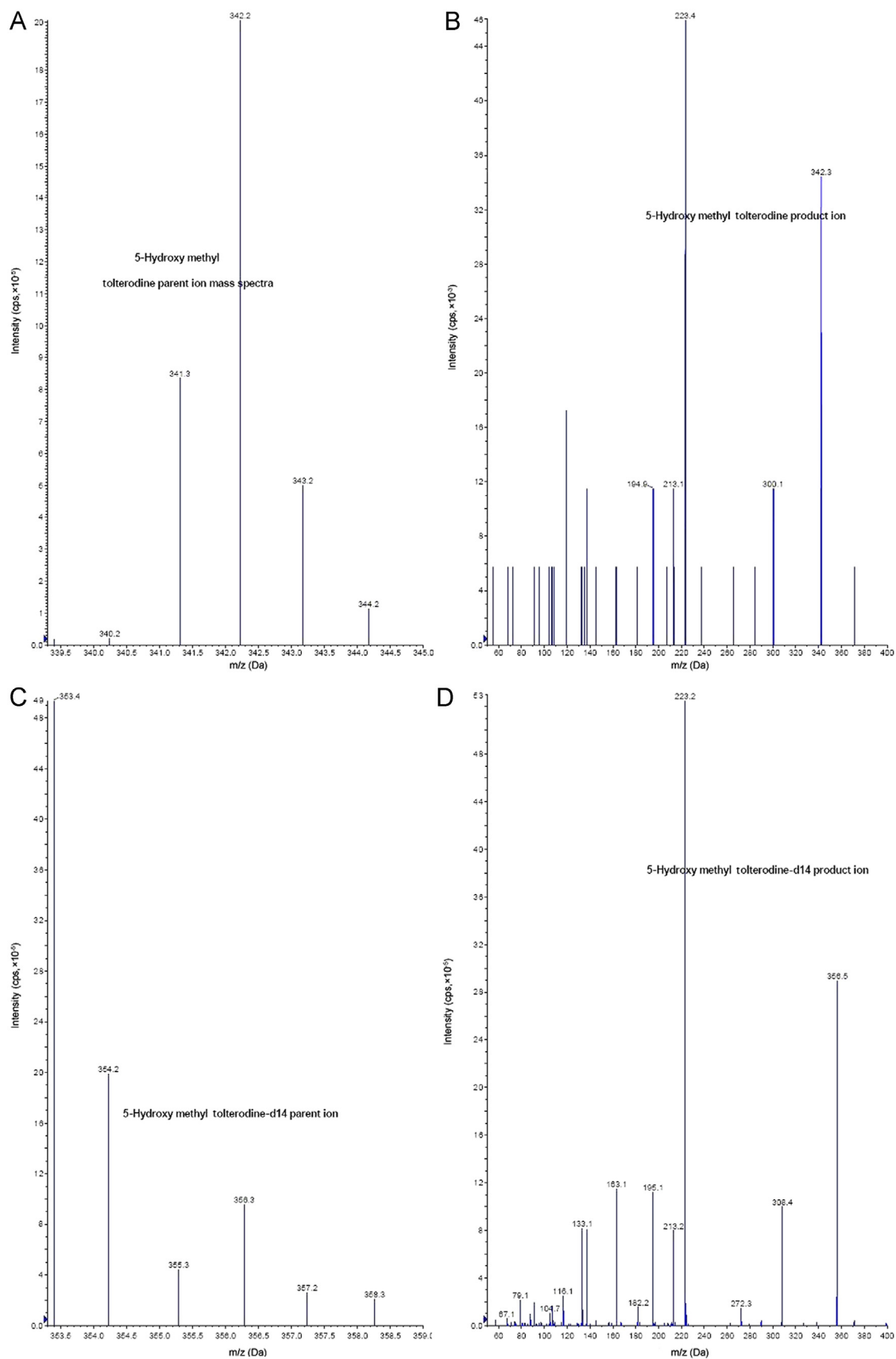


Fig. 3 Mass spectra of (A) 5-hydroxy methyl tolterodine parent ion, (B) 5-hydroxy methyl tolterodine product ion, (C) 5-hydroxy methyl tolterodine-d14 parent ion and (D) 5-hydroxy methyl tolterodine-d14 product ion.

2.7.4. Stability

Low quality control (LQC) and high quality control (HQC) samples ($n=6$) were retrieved from the deep freezer after three freeze–thaw cycles according to the clinical protocols. Samples were stored at -30°C in three cycles of 24, 36 and 48 h. In addition, the long-term stability of tolterodine/5-hydroxy methyl tolterodine in quality control samples was also evaluated by analysis after 65 days of storage at -30°C . Autosampler stability was studied following 34 h storage period in the autosampler tray with control concentrations. Bench top stability was studied for a 26 h period with control concentrations. Stability samples were processed and extracted along with the freshly spiked calibration curve standards. The precision and accuracy for the stability samples must be within ≤ 15 and $\pm 15\%$ of their nominal concentrations, respectively.

2.8. Application to pharmacokinetic study in rat plasma

The validated method has been successfully used to quantify tolterodine, 5-hydroxy methyl tolterodine concentrations in rat plasma. The study was conducted according to current GCP guidelines [26–28]. Before conducting the study it was also approved by an authorized animal ethics committee. Male Sprague–Dawley rats were obtained from Vignan College of Pharmacy, Vadlamudi, Guntur, A.P, India. After i.v. administration of 0.072 mg of tolterodine/200 g body weight of rat via left femoral vein 0.4 mL of blood samples for analytical determinations were collected via the right femoral vein at specific time

intervals (0.15, 0.25, 0.66, 1, 3, 5, 9, 17, 23 h). The blood samples were collected in separate tubes containing K_2EDTA as an anticoagulant. The plasma from these samples was separated by centrifugation at 3000 rpm within the temperature range of $2\text{--}8^{\circ}\text{C}$. The plasma samples thus obtained were stored at -30°C until analysis. After analysis the pharmacokinetic parameters were computed using WinNonlin[®] software version 5.2.

3. Results and discussion

3.1. Method development

LC–MS/MS has been used as one of the most powerful analytical techniques in clinical pharmacokinetics for its selectivity, sensitivity and reproducibility. The goal of this work was to develop and validate a simple, sensitive and rapid assay method for the quantitative determination of tolterodine, 5-hydroxy methyl tolterodine from plasma samples. A simple liquid–liquid extraction technique was utilized in the extraction of tolterodine, 5-hydroxy methyl tolterodine and tolterodine-d6, 5-hydroxy methyl tolterodine-d14 from the plasma samples. Chromatographic conditions, especially the composition and nature of the mobile phase, were optimized through several trials to achieve best resolution and increase the signal of tolterodine, 5-hydroxy methyl tolterodine and tolterodine-d6, 5-hydroxy methyl tolterodine-d14. The MS optimization was performed by direct infusion of solutions of both tolterodine, 5-hydroxy methyl tolterodine and tolterodine-d6, 5-hydroxy methyl tolterodine-d14 into the ESI source of the mass spectrometer.

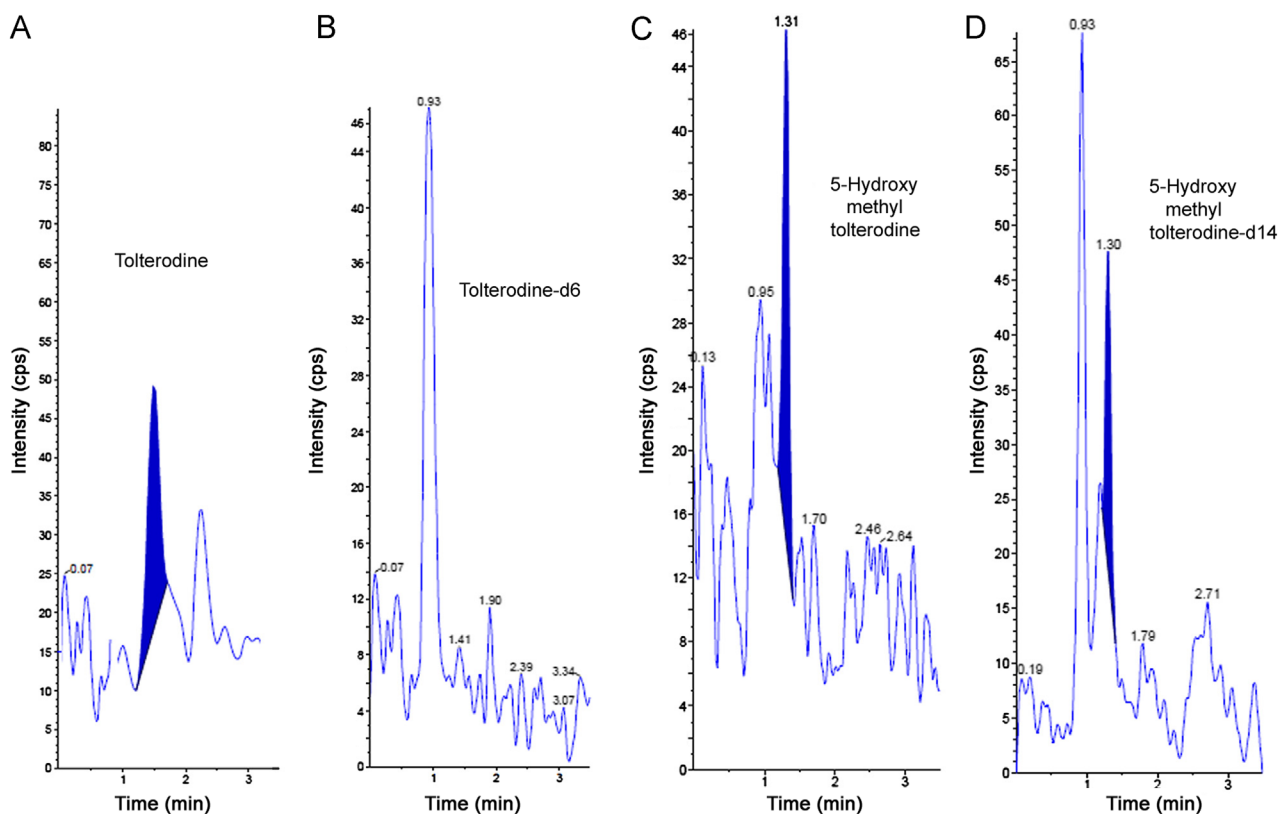


Fig. 4 Chromatogram of blank rat plasma: (A) tolterodine, (B) tolterodine-d6, (C) 5-hydroxy methyl tolterodine, and (D) 5-hydroxy methyl tolterodine-d14.

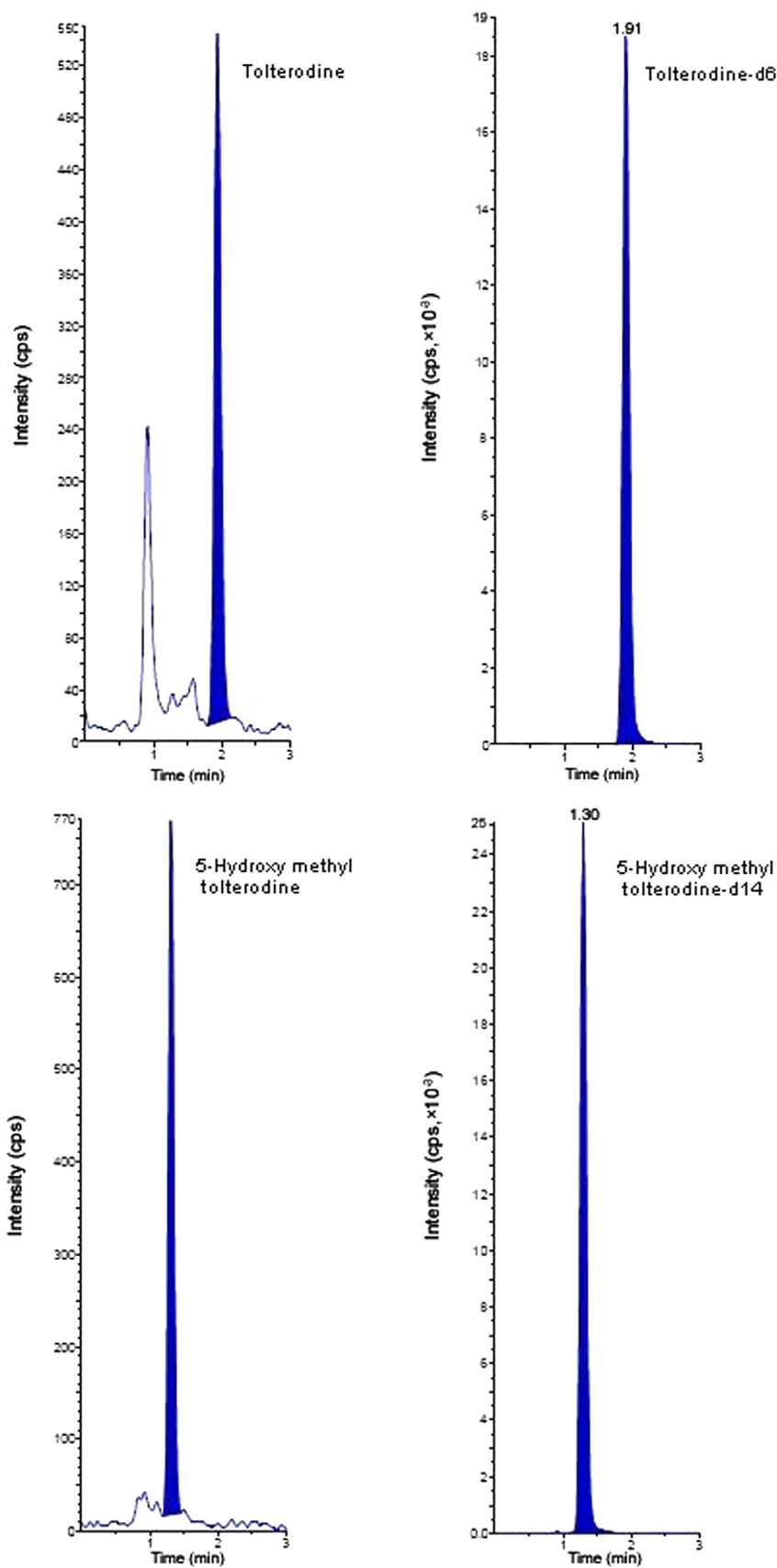


Fig. 5 Chromatogram of tolterodine, tolterodine-d6 and 5-hydroxy methyl tolterodine, 5-hydroxy methyl tolterodine-d14 at LOQ level.

Other parameters, such as the nebulizer and the heater gases, were optimized to obtain a better spray shape, resulting in better ionization. Tolterodine, tolterodine-d6, 5-hydroxy methyl tolterodine and 5-hydroxy methyl tolterodine-d14 were detected with proton adducts at m/z 326.1 \rightarrow 147.1, 332.3 \rightarrow 153.1, 342.2 \rightarrow 223.1 and 356.2 \rightarrow 223.1 in multiple reaction monitoring (MRM) positive mode respectively (Figs. 2A–D, 3A–D). After the MRM channels were tuned, the mobile phase was changed from an aqueous phase to more organic phase to obtain a fast and selective LC method. A good separation and elution were achieved using 10 mM ammonium acetate:acetonitrile (20:80, v/v) as the mobile phase, at a flow-rate of 0.5 mL/min and injection volume of 10 μ L.

3.2. Method validation^[26]

3.2.1. Specificity

The analysis of tolterodine, 5-hydroxy methyl tolterodine and tolterodine-d6, 5-hydroxy methyl tolterodine-d14 using MRM function was highly selective with no interfering compounds.

Fig. 4 shows the chromatograms of one blank rat plasma. Chromatograms obtained from plasma spiked with tolterodine (20.00 pg/mL), 5-hydroxy methyl tolterodine (20.00 pg/mL), tolterodine-d6 (6000.00 pg/mL) and 5-hydroxy methyl tolterodine-d14 (5000.00 pg/mL) are shown in Fig. 5.

3.2.2. Linearity, precision and accuracy

Calibration curves were plotted as the peak area ratio (tolterodine/tolterodine-d6) versus (tolterodine) concentration for tolterodine and the peak area ratio (5-hydroxy methyl tolterodine/5-hydroxy methyl tolterodine-d14) versus (5-hydroxy methyl tolterodine) concentration for 5-hydroxy methyl tolterodine. Calibration was found to be linear over the concentration range of 20.00–5000.00 pg/mL for tolterodine and 20.00–5000.00 pg/mL for 5-hydroxy methyl tolterodine. The precision (CV%) for tolterodine, 5-hydroxy methyl tolterodine was less than 2.26% and 3.66% respectively. The accuracy ranged from 97.36% to 101.68% for tolterodine and 97.12% to 101.62% for 5-hydroxy methyl tolterodine. The determination coefficients (r^2) for tolterodine and

Table 1 Calibration curves for tolterodine and 5-hydroxy methyl tolterodine.

Analyte	Spiking plasma concentration (pg/mL)	Concentration measured (mean \pm SD; pg/mL)	CV (%) ($n=5$)	Accuracy (%)
Tolterodine	20.00	20.08 \pm 0.19	0.96	100.40
	40.00	40.14 \pm 0.62	1.55	100.35
	100.00	98.34 \pm 1.78	1.81	98.34
	250.00	243.40 \pm 3.21	1.32	97.36
	500.00	508.40 \pm 4.83	0.95	101.68
	1000.00	1010.20 \pm 16.89	1.67	101.02
	2000.00	2028.00 \pm 31.14	1.54	101.40
	3000.00	3026.00 \pm 68.41	2.26	100.87
	4000.00	3964.00 \pm 59.41	1.50	99.10
	5000.00	4980.00 \pm 86.60	1.74	99.60
5-Hydroxy methyl tolterodine	20.00	20.18 \pm 0.36	1.77	100.90
	40.00	39.28 \pm 1.44	3.66	98.20
	100.00	100.22 \pm 1.29	1.29	100.22
	250.00	242.80 \pm 3.35	1.38	97.12
	500.00	501.20 \pm 8.58	1.71	100.24
	1000.00	1016.20 \pm 26.78	2.64	101.62
	2000.00	2016.00 \pm 35.07	1.74	100.80
	3000.00	3004.00 \pm 26.08	0.87	100.13
	4000.00	4008.00 \pm 72.94	1.82	100.20
	5000.00	5024.00 \pm 136.12	2.71	100.48

Table 2 Precision and accuracy (analysis with spiking plasma samples at four different concentrations) for tolterodine and 5-hydroxy methyl tolterodine.

Analyte	Spiking plasma concentration (pg/mL)	Inter-day			Inter-day		
		Concentration measured (mean \pm SD) ($n=6$; pg/mL)	CV (%)	Accuracy (%)	Concentration measured (mean \pm SD) ($n=6$; pg/mL)	CV (%)	Accuracy (%)
Tolterodine	20.00	19.90 \pm 1.26	6.36	99.50	19.84 \pm 0.96	4.84	99.20
	60.00	59.25 \pm 2.18	3.69	98.75	60.09 \pm 1.85	3.08	100.14
	1500.00	1553.33 \pm 18.62	1.20	103.56	1566.00 \pm 29.43	1.88	104.40
	3500.00	3616.67 \pm 22.51	0.62	103.33	3627.00 \pm 62.87	1.73	103.63
5-Hydroxy methyl tolterodine	20.00	19.73 \pm 0.83	4.22	98.67	20.07 \pm 0.85	4.25	100.35
	60.00	58.85 \pm 1.42	2.41	98.08	59.24 \pm 1.76	2.97	98.73
	1500.00	1520.00 \pm 20.98	1.38	101.33	1511.33 \pm 24.46	1.62	100.76
	3500.00	3663.33 \pm 80.42	2.20	104.67	3607.00 \pm 73.40	2.03	103.06

Table 3 Stability of the samples for tolterodine and 5-hydroxy methyl tolterodine.

Stability	Plasma concentration (pg/mL)	Tolterodine		5-Hydroxy methyl tolterodine	
		Concentration measured (mean \pm SD) ($n=6$; pg/mL)	CV (%) ($n=6$)	Concentration measured (mean \pm SD) ($n=6$; pg/mL)	CV (%) ($n=6$)
Room temperature stability (26 h)	60.00	60.67 \pm 1.29	2.13	58.88 \pm 0.45	0.77
	3500.00	3571.67 \pm 43.55	1.22	3565.00 \pm 47.64	1.34
Autosampler stability (34 h)	60.00	62.25 \pm 1.01	1.62	58.48 \pm 1.79	3.05
	3500.00	3583.33 \pm 43.20	1.21	3666.67 \pm 105.96	2.89
Freeze–thaw stability (3 cycles)	60.00	58.95 \pm 0.88	1.50	58.55 \pm 1.00	1.70
	3500.00	3576.67 \pm 52.79	1.48	3548.33 \pm 63.06	1.78
Long term stability (65 days)	60.00	60.37 \pm 1.18	1.95	59.03 \pm 0.83	1.41
	3500.00	3601.67 \pm 31.25	0.87	3543.33 \pm 42.27	1.19

5-hydroxy methyl tolterodine were greater than 0.9998 and 0.9987 respectively for all curves (Table 1). Precision and accuracy for this method was controlled by calculating the intra- and inter-batch variations of QC samples in six replicates at four concentrations (20.00, 60.00, 1500.00 and 3500.00 pg/mL) for tolterodine and 5-hydroxy methyl tolterodine as shown in Table 2. This method demonstrated intra- and inter-day precision of 0.62–6.36% and 1.73–4.84% for tolterodine, 1.38–4.22% and 1.62–4.25% for 5-hydroxy methyl tolterodine. This method demonstrated intra- and inter-day accuracy 98.75–103.56% and 99.20–104.40% for tolterodine, 98.08–104.67% and 98.73–103.06% for 5-hydroxy methyl tolterodine. These results indicate the adequate reliability and reproducibility of this method within the analytical range.

3.2.3. Stability

Quantification of tolterodine and 5-hydroxy methyl tolterodine in plasma subjected to 3 freeze–thaw (from -30°C to room temperature) cycles showed the stability of the analyte and its metabolite. The concentrations ranged from 97.98% to 100.05% for tolterodine and 99.35% to 99.44% for 5-hydroxy methyl tolterodine of the theoretical values. No significant degradation of tolterodine and 5-hydroxy methyl tolterodine was observed even after 34 h storage period in the autosampler tray and the final concentrations of tolterodine and 5-hydroxy methyl tolterodine were between 100.23% and 103.46% and 95.24% and 102.76% of the theoretical values. The room temperature stability of tolterodine and 5-hydroxy methyl tolterodine in QC samples after 26 h was also evaluated. The concentrations ranged from 99.91% to 100.83% for tolterodine and 99.91% to 99.92% for 5-hydroxy methyl tolterodine of the theoretical values. In addition, the long-term stability of tolterodine and 5-hydroxy methyl tolterodine in QC samples after 65 days of storage at -30°C was also evaluated. The concentrations ranged from 100.33% to 100.75% for tolterodine and 99.30% to 100.17% for 5-hydroxy methyl tolterodine of the theoretical values. These results confirmed the stability of tolterodine and 5-hydroxy methyl tolterodine in rat plasma for at least 65 days at -30°C (Table 3).

3.2.4. Recovery

The recovery following the sample preparation using the liquid–liquid extraction method with *t*-butyl methyl ether was calculated by comparing the peak areas of drug in plasma samples with the peak area ratios of solvent samples and was estimated at control levels of drug. The recovery values of tolterodine (at concentrations 60.0, 1500.0 and 3500.0 pg/mL) and 5-hydroxy methyl tolterodine (at concentrations 60.0, 1500.0 and 3500.0 pg/mL) were found to be

Table 4 Pharmacokinetic data for tolterodine and 5-hydroxy methyl tolterodine in rat plasma.

Pharmacokinetic parameter	Tolterodine	5-Hydroxy methyl tolterodine
C_{max} (pg/mL)	4482.08	3854.59
T_{max} (h)	0.25	0.25
AUC_{0-t} (pg h/mL)	14,582.32	12,540.83
$\text{AUC}_{0-\text{inf}}$ (pg h/mL)	14,681.03	12,625.72
$T_{1/2}$ (h)	3.25	3.25
K_{el}	0.21324	0.21324

98.32%, 91.38%, 88.35%, and 96.82%, 81.55%, 82.92%, respectively. The overall mean recovery values of tolterodine, 5-hydroxy methyl tolterodine, tolterodine-d6 and 5-hydroxy methyl tolterodine-d14 were 92.68%, 87.09%, 85.45% and 90.98% respectively.

3.2.5. LOQ and LOD

The limit of quantification LOQ for this method was proven as the lowest concentration of the calibration curve which was proven as 20.00 pg/mL for tolterodine and 5-hydroxy methyl tolterodine.

The LOD was determined using aqueous solutions. For tolterodine 10 μL of a 2.0 pg/mL solution was injected to give an on-column mass of 20.0 fg and for 5-hydroxy methyl tolterodine, 10 μL of a 0.5 pg/mL solution was injected to give an on-column mass of 5.0 fg.

3.3. Pharmacokinetics and statistical analysis

The validated method has been successfully applied to quantify tolterodine and 5-hydroxy methyl tolterodine concentrations after i.v. administration of a single-dose (0.072 mg/200 g) tolterodine in rats. The pharmacokinetic parameters evaluated were C_{max} (maximum observed drug concentration during the study), AUC_{0-23} (area under the plasma concentration–time curve measured for 23 h, using the trapezoidal rule), T_{max} (time to observe maximum drug concentration), K_{el} (apparent first order terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve, using the method of least square regression) and $T_{1/2}$ (terminal half-life as determined by quotient $0.693/K_{\text{el}}$) [27,28]. Pharmacokinetic details are shown in Table 4. The mean concentration versus time profile of tolterodine and 5-hydroxy methyl tolterodine in rat plasma is shown in Fig. 6.

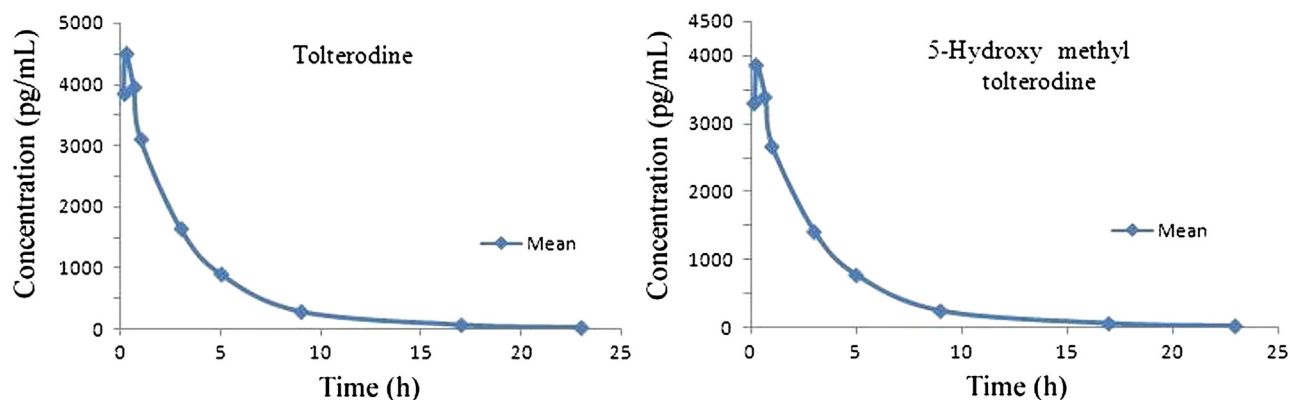


Fig. 6 Mean plasma concentrations versus time graph of tolterodine and 5-hydroxy methyl tolterodine after intravenous administration of 0.072 mg/200 g tolterodine in male rats.

4. Conclusions

In this article, we have reported the use of LC–MS/MS for the accurate, precise and reliable measurement of tolterodine and 5-hydroxy methyl tolterodine in rat plasma after i.v. administration of 0.072 mg/200 g tolterodine to healthy rats. The method described here is fast, robust, and sensitive. Each sample requires less than 3.5 min of analysis time.

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