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Pharmaceutical and Biomedical Research

Comparative *in vitro* assessment of tolterodine tartrate tablets by high performance liquid chromatographic (HPLC)

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Received: May 16, 2016, Revised: Jun 15, 2016, Accepted: Jun 21, 2016

Abstract

Tolterodine 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. The purpose of this study is to establish a reliable and quick method for the assignment of tolterodine tartrate by high performance liquid chromatography with ultraviolet detection (HPLC-UV). A rapid and sensitive high performance liquid chromatographic (HPLC) method has been developed for determination of tolterodine tartrate. Mobile phase was composed of phosphate acetate 0.1 M (pH 2.5)-acetonitrile (50:50 v/v) with a flow rate of 1.2 ml/min. The eluted peaks were detected by a UV detector was set at wavelength of 285 nm. The method was validated in the range of tolterodine tartrate concentrations from 10 to 100 μg/ml. The limits of detection (LOD) and quantitation (LOQ) of the method were 5 and 10 μg/ml, respectively. The average drug recovery was 98.20 % throughout the linear concentration range. The average within-run and between-run accuracy values of 98.56 % and 99.11 % respectively. Statistical assessment of various *in vitro* dissolution parameters and assay results was also conducted to establish if there were any significant difference among them. The validated HPLC method has been used successfully to study tolterodine tartrate.

Keywords: Tolterodine tartrate, HPLC, assay, dissolution

Pharm Biomed Res 2016; 2(2): 47-57 DOI: 10.18869/acadpub.pbr.2.2.47

Introduction

Tolterodine tartrate, (*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy- 5-methylphenyl)-3-phenyl-propanamine l-hydrogen tartrate (Fig. 1), is a new, potent and competitive muscarinic receptor antagonist in clinical development for the treatment of urge incontinence and other symptoms of unstable bladder (1-3). 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 (4). After oral administration, tolterodine is metabolized in liver, resulting in the formation of the 5-hydroxymethyl derivative, a major pharmacologically active metabolite (5). However, a small proportion

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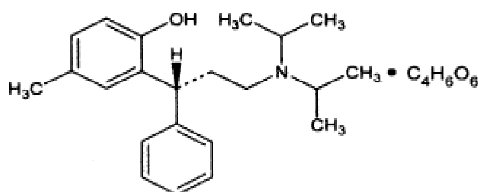

Figure1 Chemichal structure of tolterodine tartrate

of Caucasians showed a pharmacokinetic profile in accordance with poor metabolizers, having about ten time's higher tolterodine concentrations but no measurable concentrations of the 5-hydroxymethyl derivative (6-8). 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, a sensitive analytical method is needed for its determination in plasma (9). Kumar *et al.* developed an isocratic chiral HPLC method for the separation of tolterodine tartrate enantiomers but did not refer to determination in human plasma (10). Vinay *et al.* established a gas chromatography mass spectrometry (GC–MS) method to quantification of tolterodine, the limit of quantification (LOQ) in plasma of the method was only 0.5 ng/ml and it was insufficiently sensitive to enable full pharmacokinetics profiling of tolterodine tartrate (11). Swart *et al.* 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 (12); then they improved their experience with the solid-phase extraction (SPE) coupled directly to mass spectrometry (MS) method which reduced the analysis time (13). Previously, we studied bioavailability and bioequivalence of some drugs such as ezetimibe, amlodipine, atorvastatin, enalapril, cellcept by liquid chromatography mass spectrometry (LC–MS) and HPLC methods in human plasma (14-22). In this study, we describe a more simple, selective and highly sensitive method by using high performance liquid chromatography for the determination of tolterodine tartrate in human plasma.

Materials and methods

Materials

Tolterodine tartrate extended release test tablets (batch no. 014, Tehran- darou), tolterodine tartrate reference tablets (batch no. N771F,) and tolterodine tartrate reference standard (99.9% purity) were supplied and identified by Pharmacia, Upjohn (Italy). 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 re distillation before use.

Instrument and HPLC method

The HPLC system to include of pump (KNAUER, model 1000, Germany), wavelength UV detector (KNAUER, model 2800(DAD), Germany) used at a wavelength of 285 nm with the outputs to record and analyze using with a software (Chrome Gate, KNAUER, Germany). The

drug analization was performed using a C18 analytical column (250 mm × 4.6 mm, particle size 5 μm; Perfectsill, MZ-Analysente chnik, Germany) equipped by a guard column of the same packing. The mobile phase was composed of acetate buffer (pH 2.5)-acetonitrile (50:50 v/v) with a flow rate of 1.2 ml/min. Sample injection to system (50 μL) was made by a loop injector (Rheodyne®7725i, Cotati, CA, USA).

Preparation of stock solutions

Stock solutions of tolterodine tartrate was prepared in HPLC mobile phase at concentrations of 1 mg/ml and were stored at 4 °C.Working solutions of tolterodine tartrate were prepareddaily in HPLC mobile phase by appropriate dilution at 5.0,10.0, 25.0, 50.0, 100.0, 250.0, 500.0, 1000, and 1500 μg/ml.

Estimation of tolterodine tartrate in tablet dosage form

Each tablet contains 2 mg of tolterodine tartrate. Twenty tablets were taken and weighed accurately. The average weight of one tablet was calculated and powdered. Equivalent to 2 mg of tolterodine tartrate of powder was taken and transferred to a 100 mL volumetric flask and about 75 mL of phosphate buffer at pH 1.2 was added and sonicated to dissolve. The volume was made up to the mark with phosphate buffer. The solution was filtered through a membrane filter (0.22 μm) and sonicated to degas. Then 5 mL of above solution was pipetted out in 50 mL volumetric flask and volume was made up to the mark with phosphate buffer. The prepared solution was injected into the HPLC system and the observation was recorded.

Dissolution test

The dissolution test was undertaken using tablet dissolution tester in 6 replicates for each brand. Dissolution media were USP buffer solutions at pH 1.2 (phosphate buffer solution). The medium was maintained at 37 ± 0.5 °C. In all the experiments, 5 mL of dissolution sample was withdrawn at 0, 10, 20, 30, 40, 50 and 60 min and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by HPLC method. The concentration of each sample was determined from a calibration curve obtained from pure samples of tolterodine tartrate.

Method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, stability, detection limit and quantization limit according to the principles of the FDA industry guidance (23-30).

.Assay specificity

To evaluate the matrix effect on the ionization of analyses, five different concentration levels of tolterodine tartrate (5, 10, 20, 30, 40, 50, and 100.0 μg/ml) were prepared in the drug-free blank plasma as five sample series using five different lots of the drug-free plasma and the samples were processed, as described, and injected to HPLC. Standard curves of ten concentrations of tolterodine tartrateranged 10–100.0 μg/ml were assayed. The limit of

detection (LOD) was estimated from the signal-to- noise ratio. This parameter was defined as the lowest concentration level resulting in a peak area of three times the baseline noise.

Precision and accuracy Within-run variations

In one run, three samples with concentrations of 10, 50, and 100 µg/ml (from high, middle, and low regions of the standard curve) were prepared in triplicate and analyzed by developed HPLC method. Then, the coefficient of variations (%CV) of the corresponding determined concentrations were calculated in each case.

Between-run variations

On three different runs, samples from upper, intermediate, and lower concentration regions used for construction of standard curve were prepared and analyzed by HPLC method. Then, the corresponding %CV values were calculated.

Extraction recovery

Three samples with concentrations of 10, 50, and 100 µg/ml (from high, middle and low regions of the standard curve) were prepared in triplicate and analyzed by developed HPLC method. Then, the ratio of the recorded peak heights to the peak heights resulted from the direct injection of the aqueous solutions of toltrodine with the same concentrations were determined as percentage in each case.

Stability

Freeze and thaw stability: Three concentration levels of QC samples were stored at the storage temperature (−20 ºC) 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 ℃)-thaw (room temperature).

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

Long-term stability: Three concentration levels of QC samples kept at low temperature (−20 ^{0} C) were studied for a period of 4 weeks.

Post-preparative stability: The auto sampler stabilitywas conducted reanalyzing extracted QC samples keptunder the auto sampler conditions (4 ℃) for 12 h.

Results

Method development

In response to lack of an accessible, consistent, and simpletouse analysis method for tolterodine tartrateassay in plasma as an vital part of pharmacokinetic and bioequivalence estimate projects on the drug we urbanized a simple and offered HPLC method with UV detection based on the available equipments found in most pharmaceutical laboratories. To this end, initially a series of isocratic as well as gradient conditions using different usual mobile phase compositions, polarities, ionic strengths, and pH values were tested in order to determine the best condition for the analyte separation.

System suitability tests

The number of theoretical plates (N), peak symmetry, and retain ability (K') of the method for tolterodine tartratewere 1296, 1.143, and 2.75, respectively. These data show that the developed method is of appropriate separation efficiency and peak shape, both of which are important factors in estimate of the chromatographic method outputs. Typical chromatograms produced from the developed method are shown in figure 2. Figure 2.A shows are presentative chromatogram of a sample obtained at 7 h from a subject who received a single oral dose (2 mg), no interferences of the analyst were observed and the retention times of tolterodine tartratewas 4.98 min and the total HPLC analysis time was 7 min per sample. The HPLC chromatogram for a blank sample indicating no endogenous peaks at the retention positions of tolterodine tartrate was shown in figure 2.B.

Linearity

The method produced linear responses throughout the tolterodine tartrate concentration range of 10-100μg/ml, which is suitable for intended purposes. A typical linear regression equation of the method was: $y = 7.345\ x + 0.0234$, with x and y representing tolterodine tartrateconcentration (in mcg/ml) and peak height (in arbitrary units), respectively, and the regression coefficient (r^2) of 0.9942. The lower limit of quantification for tolterodine tartratewas proved to be 10 μg/ml and the lower limit of detection was 5 μg/ml. Figure 2.B shows the chromatogram of an extracted sample that contained 20 μg/ml of tolterodine tartrate.

Within-run variations and accuracy

The within-run variations of the developed HPLC method as well as the corresponding absolute recoveries are shown in table 1.

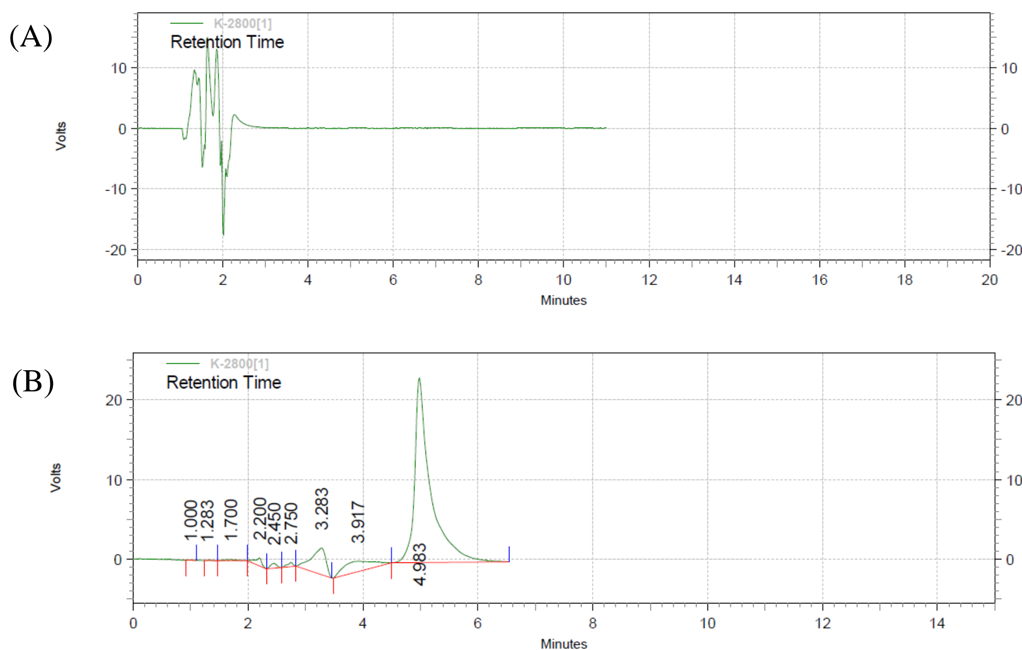

Figure2 Chromatogarm of samples: A: Blank B: chromatogram of an extracted sample that contained 20 μg/ml of tolterodine tartrate.

Between-run variations and accuracy

The between-run variations of the developed HPLC method as well as the corresponding absolute recoveries are shown in table 2.

Table 1 Within–run variations and accuracy of the LC-Mass method for quantitation of Tolterodine (n = 3).

Nominal added concentration (μg/ml)	Sample number	Mean ± SD
10	1 2 3	9.91 ± 0.015
50	1 2 3	50.13 ± 0.23
100	1 2 3	100.01 ± 0.077

Table 2 Between–run variations and accuracy of the HPLC method for quantitation of tolterodine (n = 3)

Nominal added concentration (μg/ml)	Run number	Mean ± SD
10	1 2 3	10.1 ± 0.015
50	1 2 3	49.99 ± 0.17
100	1 2 3	100.16 ± 0.084

Extraction recovery

The extraction recovery determined for tolterodine tartrate was shown to be consistent, precise and reproducible. Data was shown below in table 3.

Stability

Table 4 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.

Estimation of tolterodine tartrate in tablet dosage form

The percent content of tolterodine tartratein tablet dosage form was found to be 100.25 %. The USP specifications for assay are that the tolterodine tartratecontent should be less than 90 % and not more than 110 %. Therefore, the assay results ascertain the presence and compendia quality of tolterodine tartrate in all these products (Table 5).

In vitro drug release study

The release profiles of different brands of tolterodine tartrate tablets are shown in figure 3 and table 6 , 7. All dissolution data are based on the actual drug content of the test tablets as calculated from the assay results. Around 80% drug was released

Table 3 Relative recovery of Tolterodine by the HPLC method (n = 3)

Nominal added concentration (µg/ml)	Sample number	Mean ± SD
10	1 2 3	97.21 ± 1.84
50	1 2 3	94.18 ± 1.58
100	1 2 3	96.67 ± 0.35

Table 4 Data showing stability of tolterodine tartrate at different QC levels (n = 5)

	10(µg/ml) Mean ± SD	50(µg/ml) Mean ± SD	100(µg/ml) Mean ± SD
Short-term stability	95.21 ± 2.12	94.65 ± 4.76	95.68 ± 6.21
Freeze and thaw stability	97.56 ± 3.98	96.23 ± 3.12	96.63 ± 3.65
Long-term stability	93.68 ± 2.21	97.65 ± 1.54	97.32 ± 4.32
Post-preparative stability	91.32 ± 1.87	92.21 ± 2.54	92.21 ± 1.12

Table 5 Assay of tolterodine tartrate

Number	1	2	3	4	5	6	7	8	9	10	Mean ± SD	RSD
Percentage of label claimed	99.7	104.5	102.4	101	102.6	102.6	103.5	104.8	103.5	104.5	100.25 ± 1.6	1.57

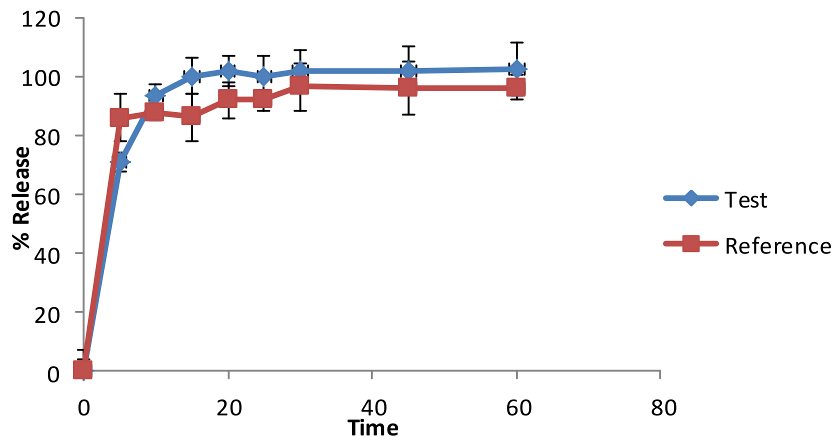

Figure 3 Diagram dissolution of tolterodine tartrate for test and reference sample

Table 6 Dissolution of tolterodine tartrate for test sample

Time (min)	1	2	3	4	5	6	Mean ± SD	SD	RSD
5	68.07	89.24	42.09	49.22	90.80	85.76	70.86 ± 21.27	21.27	30.01
10	106.54	97.27	79.25	85.87	95.85	98.49	93.88 ± 9.75	9.75	10.38
15	103.43	100.49	106.09	97.82	95.38	98.92	100.36 ± 3.89	3.89	3.87
20	98.02	101.18	117.96	99.82	96.00	99.67	102.11 ± 7.97	7.97	7.80
25	103.39	95.33	106.79	100.11	96.64	100.06	100.39 ± 4.24	4.24	4.22
30	106.99	99.26	107.59	100.87	97.32	100.96	102.16 ± 4.19	4.19	4.10
45	107.58	99.08	103.60	103.51	96.44	103.06	102.21 ± 3.91	3.91	3.82
60	111.41	98.95	101.21	102.12	98.49	104.09	102.71 ± 4.74	4.74	4.61

Table 7 Dissolution of tolterodine tartrate for reference sample

Time (min)	1	2	3	4	5	6	Mean ± SD	RSD
5	96.53	89.90	51.46	92.35	93.80	92.77	86.13 ± 17.12	19.88
10	105.60	92.98	45.94	94.71	94.45	95.12	88.13 ± 21.17	24.02
15	92.41	89.40	47.20	98.32	96.67	95.26	86.54 ± 19.53	22.57
20	100.97	88.27	69.35	99.70	98.18	97.00	92.25 ± 12.08	13.09
25	92.89	92.29	69.26	101.44	99.52	98.20	92.27 ± 11.85	12.84
30	99.32	85.93	86.98	102.22	105.61	100.06	96.69 ± 8.23	8.51
45	100.79	85.30	81.97	104.08	103.92	100.59	96.11 ± 9.83	10.23
60	100.55	94.34	80.53	103.81	97.24	102.32	96.46 ± 8.53	8.84

within 30 min and almost 100% drug was released within 60 min from all the brands in phosphate buffer.

Discussion

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 (1-3). Several bioanalytical methods are reported to determine tolterodine tartrate in different biological matrices like plasma, serum, urine, and cerebrospinal fluids (6-8). Although these methods were sufficiently sensitive, they were not suitable for most laboratories to perform studies involving samples in high through-put for therapeutic monitoring (9). In the present study, we describe a simple, selective and high-through put method using HPLC for the estimation of tolterodine tartrate. The method was fully validated and applied successfully to quantify the drug in pharmaceutical dosage form. A short chromatographic run time of 7 min allows the quantification of tolterodine tartrate in bulk raw material, tablet dosage form in quality control laboratories, and is compatible with LC-MS technique where there is no need for traditional HPLC methods with complex mobile phase mixtures, long chromatographic run times and more solvent consumed methods. The developed RP-HPLC technique will eliminate significant time and cost per sample from analytical process while improving the quality of results. Statistical

assessment of various *in vitro* dissolution parameters and assay results was also conducted to establish if there were any significant difference among them. The validated HPLC method has been used successfully to study tolterodine tartrate. The validation tests on the developed method showed acceptable degree of linearity, sensitivity, precision, accuracy and recovery for the method.

Conclusion

A sensitive, selective, accurate and precise HPLC method was developed and validated for determination of tolterodine tartrate. The reported method offers several advantages makes the method suitable for the analysis of large sample batches resulting from the pharmacokinetic, bioavailability or bioequivalent study of tolterodine tartrate.

Acknowledgement

The authors would like to thank the authority of the Faculty of Pharmacy, Zanjan University of Medical Sciences, for their support. The authors wish to thank Tehran Darou Pharmaceuticals (Tehran, Iran) for kindly providing us by tolterodine tarterate samples.

Conflict of interest

The authors declared no potential conflict of interest with respect to the authorship, and/or publication of this study.

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