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An enantiospecific HPLC method for the determination of (*S*)-enantiomer impurities in (*R*)-tolterodine tartarate

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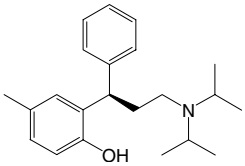
A high-performance liquid chromatographic method was developed for the separation of the enantiomers of tolterodine tartarate. The proposed method was applied to the determination of (*S*)-isomer in (*R*)-tolterodine tartarate, and satisfactory results were obtained. The enantiomers of tolterodine tartarate were separated on a Chiralpak AD-H (250 mm × 4.6 mm) column containing amylose tris-(3,5-dimethyl-phenyl-carbamate) at room temperature. The mobile phase consisted of *n*-hexane and isopropyl alcohol in the ratio of 85 : 15 (v/v) with 0.075% triethylamine (TEA) and 0.05% trifluoroacetic acid (TFA) as the additive. The flow rate was kept at 0.5 ml/min, and UV detection wavelength was set at 283 nm. The calibration curves of (*S*)-enantiomer in the concentration range from 0.05 μg/ml to 1 μg/ml range were linear. The relative standard deviations of within-day and between-day were less than 2% (n = 3). The limit of detection (LOD) was 0.75 ng (S/N = 3) and the limit of quantification (LOQ) was 0.05 μg/ml (RSD < 4.1%, n = 3). The determination recoveries of the (S)-enantiomer were in the range of 98.2–104.8%. The results demonstrated that the developed HPLC method was a reliable, simple technique and was applicable to the purity determination of (R)- tolterodine tartarate.

1. Introduction

Tolterodine [(*R*)-N,N-diisopropyl-3-(2-hydroxy-5-methyl-phenyl)-phenylpropanamine] is a new muscarinic receptor antagonist that was specifically developed for the treatment of urinary urge incontinence and other symptoms associated with an overactive bladder (Stigh et al. 1998). The pharmacological activity of the (*R*)-enantiomer is that of the 100 times (*S*)-enantiomer (Huang Zong-yu et al. 2001). The asymmetric reaction was applied to the synthesis of (*R*)-tolterodine (Chen et al. 2005), which would unavoidably inlet some (*S*)-enantiomer. So a simple and effective analytical method was required that could allow enantiomeric separation for the purity control of tolterodine tartarate and other studies.



(*R*)-Tolterodine

2. Investigations and results

2.1. Separation parameters

Good chromatography parameters were obtained, with the resolution of 2.9 between the peaks of the (*R*)-tolterodine tartarate and (*S*)-isomer, and a number of the theoretical plates of the column more than 7000. The results are shown in Table 1.

The chromatograms of (*R*)-and (*S*)-isomers and their mixture are displayed in Fig. 1. (*S*)- and (*R*)-enantiomers were separated completely under the chromatographic conditions employed.

2.2. Standard curve and sensitivity of the method

A quantity of (*S*)-tolterodine was dissolved and diluted with mobile phase to 1, 0.5, 0.25, 0.125, 0.05 μg/ml for construction of the standard curve. The regression equation was $y = 32879x + 62.966$, $R^2 = 0.9997$ (Fig. 2).

A standard addition method was employed for checking the measured error of (*S*)-isomers when (*R*)-tolterodine tartarate was present in test solution. Accurately measured (*R*)-tolterodine stock solution was added to different volumes of the (*S*)-tolterodine reference solution to produce a series of mixtures (containing 1, 0.5, 0.25, 0.125, 0.05 μg/ml

Table 1: System suitability results

	$W_{1/2}$	T	N	k	α	Rs
(*R*)-tolterodine tartarate	0.52	1.13	7257	1.79	1.22	2.9
(*S*)-tolterodine	0.45	1.06	7780	2.19		

$W_{1/2}$: Half-width, T: Tailing factor N: Number of theoretical plates α: Separation factor. Rs: Resolution k: retention factor

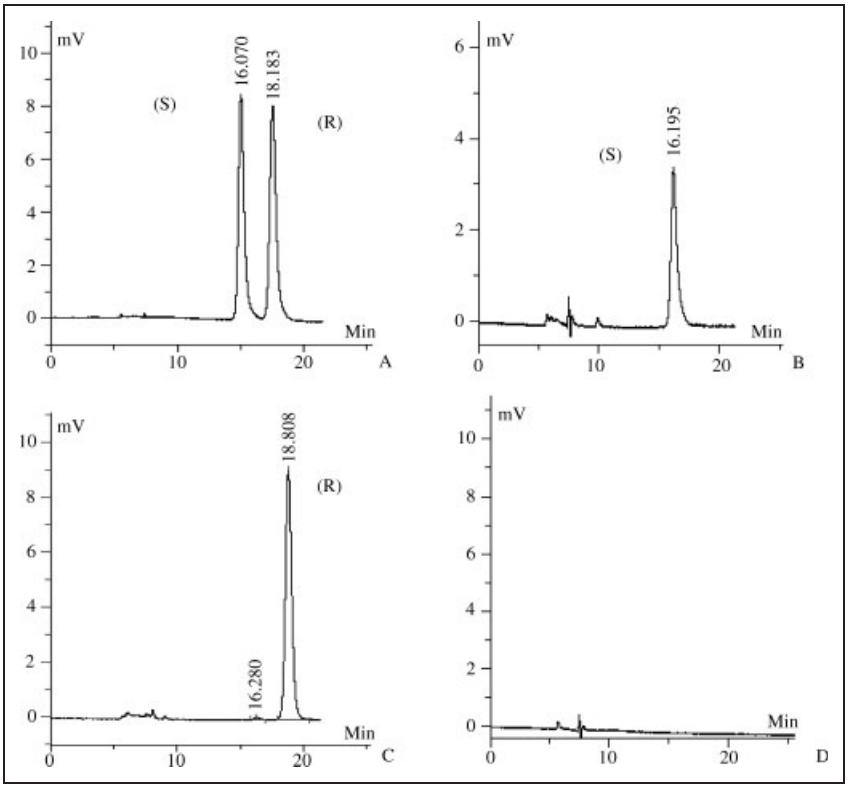


Fig. 1:
The Chromatograms of individual and mixture of (*R*)-and (*S*)-isomers
A. mixture of (*R*) -and (*S*)-isomers; B. (*S*)-isomer; C (*R*)-isomer; D. solvent blank

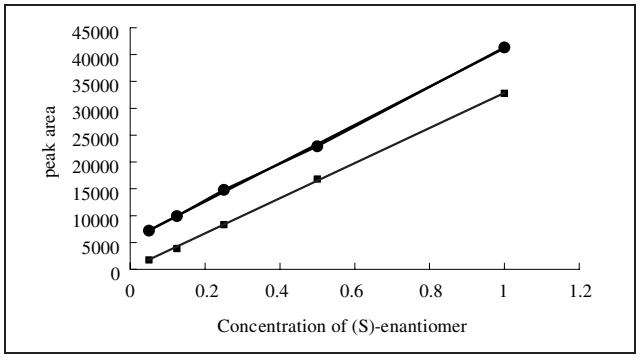


Fig. 2: Calibration curves by general external standard method (■) and by standard addition method (●)

(*S*)-isomer and 25 μg/ml (*R*)-tolterodine tartarate). The solutions (20 μl) were injected into the HPLC system and the chromatograms were recorded. The calibration curves of (*S*)-tolterodine, under the presence of (*R*)-tolterodine tartarate, was constructed by plotting the peak area (y) vs. concentration (x) of (*S*)-tolterodine in the mixtures. The regression equation was $y = 35716x + 5484.9$, $R^2 = 0.9996$ (Fig. 2).

The two calibration curves of (*S*)-enantiomer in Fig. 2 were essentially parallel. The curve slope received by general external standard method ($b = 32879$) was 8% less than that received by the standard addition method ($b = 35716$).

The limit of detection (LOD) for (*S*)-tolterodine was 0.75 ng (signal-to-noise ratio 3) with step by step dilute method. The limit of quantification (LOQ), defined as the lowest concentration of (*S*)-tolterodine that could be quantitatively determined with suitable precision and accuracy, was 0.050 μg/ml (RSD $< 4.1\%$, $n = 3$) by the external standard method.

2.3. Recovery and repeatability

The intra- and inter-day precision and accuracy were obtained by analyzing the spiked samples at concentrations of 0.0875, 0.25 and 0.75 μg/ml in triplicate within one day and at three consecutive days, respectively. The results are shown in Table 2. The recoveries of (*S*)-enantiomers at different concentration were assessed from the regression equation obtained by standard addition method. The method recoveries were 98.2%–104.9% for (*S*)-tolterodine. The results are shown in Table 3.

Table 2: Precision, accuracy for the assay of (*S*)-enantiomers, n = 3

Spiked amount (*S*)-enantiomers (μg/ml)	Intra-day RSD (%)	Inter-day RSD (%)
0.0875	2.0	2.3
0.25	0.6	0.9
0.75	0.3	1.3

Table 3: Recoveries of (*S*)-enantiomers from standard addition method, n = 3

Spiked amount (*S*)-enantiomers (μg/ml)	Measured amount (*S*)-enantiomers (μg/ml)	Recoveries %	RSD %
0.0875	0.0859	98.2	1.9
0.25	0.2528	101.1	0.9
0.75	0.7864	104.9	1.2

2.4. Determination of three batch samples

The *S*-enantiomer impurities in three batch of (*R*)-tolterodine tartarate samples were determined by the developed method, and the results are shown in Table 4. The contents of *S*-enantiomer in three batches sample examined were all less than 1%, no matter if calculated by the external standard method or the standard addition method.

3. Discussion

According to literature reports, Chiralcel OD and Chiralpak AD columns provide different retention, separation

Table 4: Determination of (*S*)-enantiomer impurities in (*R*)-tolterodine tartarate sample, n = 3

Batch No.	(*S*)-Enantiomer content (%) ± SD		
	051001	050901	050902
Content in external standard method	0.62 ± 0.019	0	0.15 ± 0.006
Content in standard addition method	0.67 ± 0.017	0	0.24 ± 0.006

and elution order of some enantiomeric pairs (Yu Lushan and Yao Tongwei 2003; Wang et al. 2000). It had been assumed that the separation of enantiomers on these cellulose- and amylose-based CSPs was due to the formation of solute-CSP complexes through inclusion of the enantiomers into the chiral cavities in the higher order structures of the CSPs (Okamoto and Kaida 1994; Francotte et al. 1985; Wainer et al. 1987). Kumar et al. (2004) established an HPLC method to determine the (*R*)-tolterodine tartarate with Chiralcel OD. Under their chromatography conditions, the retention time of (*S*)-tolterodine was langer than that of (*R*)-tolterodine tartarate, and the resolution was only 2.5, which was not enough for (*S*)-isomer impurity control, because the minute peak of trace (*S*)-isomer was easily hidden by the peak of (*R*)-tolterodine tartarate. In our research, the peak of (*S*)-tolterodine was at the front of the (*R*)-tolterodine tartarate peak, which was advantageous to detect the (*S*)-isomer.

We found that the additives and modifiers in the mobile phase (*n*-heptane was used as mobile phase) played an important role in the separation and elution sequence of tolterodine enantiomers. Isopropanol or ethanol were chosen as organic solvent modifiers of the mobile phase and the chromatograms were compared.

The results displayed elution sequences of both enantiomers were different under different modifiers conditions. The (*S*)-isomer was firstly eluted when using isopropyl alcohol as modifier, on the contrary, when using ethanol in the microamount impurity test, it was possible that the impurity peak was overlaid by the main peak tailing if the impurity peak was at the back of the main component peak, which was unfavourable for impurity detection. And the phenomenon would not occur if the impurity peak was in front of the main peak. So we chose isopropanol as the organic modifier.

The acidic and basic additives in the mobile phase were essential for the separation of the two isomers of tolterodine. The preliminary experiments carried out with only *n*-heptane and isopropanol/ethanol in different ratio were not successful in the separation of these isomers. And the separation was also not achieved if only trifluoroacetic acid or triethylamine was added to the mobile phase. However, the separation of both isomers was achieved when trifluoroacetic acid and triethylamine coexisted in the mobile phase in a suitable concentration, due to the amphotericity of tolterodine tartarate.

Different concentrations of the trifluoroacetic acid (from 0.02% to 0.09%) in the mobile phase were compared under fixing 0.05% triethylamine. An interesting phenomenon was observed. The resolution between two enantiomer peaks increased when the quantity of acid increased from 0.02% to 0.05%, but decreased when the amount of acid continued increasing. So 0.05% trifluoroacetic acid was chosen, then the quantity of triethylamine compared. The resolution between the enantiomer peaks was gradually increasing, following the increasing percentage of triethylamine in the mobile phase from 0.05% to 0.1%, but this increasing tendency was not significant when the concentration of triethylamine exceeded 0.075%. So 0.075% triethylamine was chosen.

4. Experimental

4.1. Reagents and apparatus

HPLC-grade *n*-hexane, isopropanol alcohol and ethanol were procured from TEDIA company lnc; HPLC-grade methanol was obtained from The Chemical Reagent Factory of ShangHai Ludu; trifluoroacetic acid was procured from Sinopharm Group Chemical Reagent Co.,Ltd; triethylamine was purchased from ShangHai Chemical Reagent Purchase Provision Wulian Chemical Industry. (*S*)- and (*R*)-isomers of tolterodine tartarate were provided by Zhejiang Xinhua Pharmaceutical Co., Ltd. All other chemicals were of analytical grade and obtained from common commercial sources. All studies were performed on a Shimadzu HPLC system: LC-6A Liquid chromatograph, SCL-6A system controller, SPD-6AV UV-vis spectrophometric detector and HPLC System Manager software, HS2000. The chiral seperation was carried out on ChiralpakAD-H (250 mm × 4.6 mm i.d., 5 μm particle size.), purchased from Diacel Chemical Industries Ltd., Japan.

4.2. Chromatography

Mobile phases were prepared by mixing the indicated volumes of *n*-hexane, isopropyl alcohol, trifluoroacetic acid and triethylamine (85 : 15 : 0.05 : 0.075, v/v/v/v), and filtered through a 0.45-μm Millipore filter and degassed in an ultrasonic bath. Flow rate was kept at 0.5 ml/min. The sensitivity of the detector was 0.005 AUFS, and UV detection wavelength was at 283 nm. The injecting volume was 20 μL.

4.3. Methods

(*S*)- or (*R*)-isomer of tolterodine tartarate was dissolved in the mixing solvent of methanol-isopropanol (4 : 6) in a concentration of about 1 mg/ml, as the stock solution. An accurately measured quantity of the (*R*)-tolterodine tartarate stock solution, was diluted with mobile phases to produce the test solution of about 25 μg per ml. The (*S*)-tolterodine reference solution was prepared by diluting accurately a quantity of (*S*)-tolterodine tartarate stock solution with the mobile phase to about 8 μg/ml. An accurately measured quantity of (*S*)-and (*R*)-tolterodine stock solution, was mixed well, diluted with mobile phase to produce a mixed solution containing 12.5 μg/ml of (*S*)- and (*R*)-tolterodine each.

Aliquots of 20 μL (*S*)-tolterodine reference solution and mixed solution were injected into the HPLC system the retention time of the (*S*)-tolterodine was determined and the chromatograms were recorded. The theoretical plates of the column and the resolution between (*S*)-and (*R*)- tolterodine were calculated.

An aliquot of 20 μl test solution of (*R*)-tolterodine was injected into the HPLC system and the chromatogram was recorded. The peak area of the (*S*)-isomer impurity in the (*R*)-tolterodine test solution was measured. The content of the (*S*)-isomer was calculated by the standard curve method.

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