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Short communication

A validated chiral HPLC method for the enantiomeric separation of tolterodine tartarate

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Abstract

An isocratic chiral HPLC method was developed for the separation of tolterodine tartarate enantiomers. The mobile phase consists of *n*-hexane and isopropyl alcohol in the ratio of 980:20 (v/v) with 1 ml diethylamine and 0.6 ml trifluoroacetic acid. Chiralcel OD-H (250 mm × 4.6 mm) column was used at constant room temperature. Flow rate was kept at 0.5 ml/min. This method is capable of detecting the *S*-isomer up to 0.1 μg/ml. The method was validated in terms of linearity, precision, limit of detection (LOD) and limit of quantification (LOQ).

Keywords: Tolterodine tartarate; Enantiomers; Chiral HPLC

1. Introduction

The determination of the stereoisomeric composition of pharmaceuticals is rapidly becoming one of the key issues in the development of new drugs. Among the methods currently used to achieve chiral separation of racemic mixtures, high resolution liquid chromatography systems based on chiral stationary phases (CSPs), (direct methods) are more rapid and suitable for the resolution of racemic mixtures of pharmacologically active chemical entities [1–4].

Tolterodine is a cholinergic antagonist for the treatment of urinary incontinence. It is a follow up to terodiline, which was withdrawn due to side effects. Tolterodine has a safety profile in humans with no side effects. In the search for new drugs with improved side effect profiles to treat an overactive bladder, a family of phenyl propyl amines as muscarainic receptor antagonists has been investigated and very recently, (+)-*N*, *N*-diisopropyl-3-(2-hydroxy-5-methyl phenyl)-3-phenyl propylamine ((+) (*R*) tolterodine, brand names Detrol or Detrugitol) was

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launched and marketed world wide with success as (*R*,*R*)-tartaric acid salt. A convenient synthesis for this drug was cited in the literature [5] starting from 1-[2-hydroxy-5-methyl) phenyl]-1-phenyl ethylene, accessible in high yield by alumina-promoted ortho alkenylation of *p*-cresol with phenyl acetylene. The enantiomeric purity of tolterodine tartarate is very important issue for quantitative estimation of enantiomers of tolterodine.

As per our knowledge till date there was no reported validated method in the literature for the chiral separation of tolterodine tartarate.

2. Experimental

2.1. Equipment

A Waters Model Alliance 2690-separation module equipped with an autosampler and waters 996-photo diode array UV detector was used for analysis. The analysis was carried out on Chiralcel OD-H column, 250 mm × 4.6 mm i.d., 5 μm particle size (Diacel Chemical Industries Ltd., Japan). The data was recorded using Waters Millennium software.

2.2. Chemicals

HPLC-grade hexane and isopropyl alcohol were procured from Ranbaxy fine chemicals Ltd. (India). Diethylamine and trifluoroacetic acid was procured from Fluka. (*S*) and (*R*) isomers of tolterodine tartarate were obtained from process development laboratory of Dr. Reddy's Laboratories Ltd., Bulk Actives unit-III.

2.3. Sample preparation

Fifty milligrams of *R*-tolterodine tartarate was dissolved in 25 ml of water and basified the solution with 2N NaOH and transferred the solution in to separating funnel. This solution was extracted with hexane and washed twice with water. This solution was injected after drying over 1 g of sodium sulfate. This sample preparation procedure has been developed due to poor solubility of tolterodine tartarate in the mobile phase. This procedure is found to be reproducible with the concentration of 0.3 μg/ml.

3. Results and discussion

The preliminary trials carried out in reverse phase chiral columns were not fruitful in the separation of these isomers. The separation was not achieved in presence of diethylamine and trifluoroacetic acid alone with the combination of hexane and isopropyl alcohol. However, the resolution for both the isomers was achieved only with the presence of these two only. Moreover, the quantity of trifluoroacetic acid is found to be very crucial in the separation of these isomers. Different experiments have been conducted by varying the quantity of trifluoroacetic acid and found the interesting observations. If the quantity of acid decreased from 0.6 ml for 1000 ml mobilephase to 0.5, 0.4, 0.3 and 0.2 ml the retention time decreased drastically and the resolution too. On the other hand if we increase the amount of acid to 0.7 ml the elution time increased to 40–50 min. The separation for these isomers was tried with the combination of triethyl amine and acetic acid with hexane and 2-propanol combination also with Chiralcel OD-H column. But the separation was found to be very selective with respect to the acid presence. Finally, the separation was achieved only with 0.6 ml trifluoroacetic acid and 1 ml diethylamine with the mobile consisting of *n*-hexane and isopropyl alcohol in the ratio of 980:20 (v/v). Chiralcel OD-H (250 mm × 4.6 mm) column was used at constant room temperature. Flow rate was kept at 0.5 ml/min. The elution was monitored at wavelength $\lambda = 284$ nm. The resolution was found to be as 2.5 for the separation of these two isomers.The structures of *R* and *S* isomers of tolterodine tartarate are displayed in Fig. 1. The chromatograms of individual and mixture of *R* and *S* isomers are displayed in Figs. 2–4, respectively.

As per the ICH guidelines the method was validated in terms of following parameters.

3.1. Linearity and accuracy

Standard solutions were prepared as per the sample preparation described in Section 2.3 with the concentrations between 0.1 and 0.6 mg/ml. The calibration curve was drawn between the peak area of (*R*) isomer versus its concentration. The accuracy of method was evaluated by assaying freshly prepared solutions in triplicate at the concentrations of 0.15, 0.25, 0.35 mg/ml.

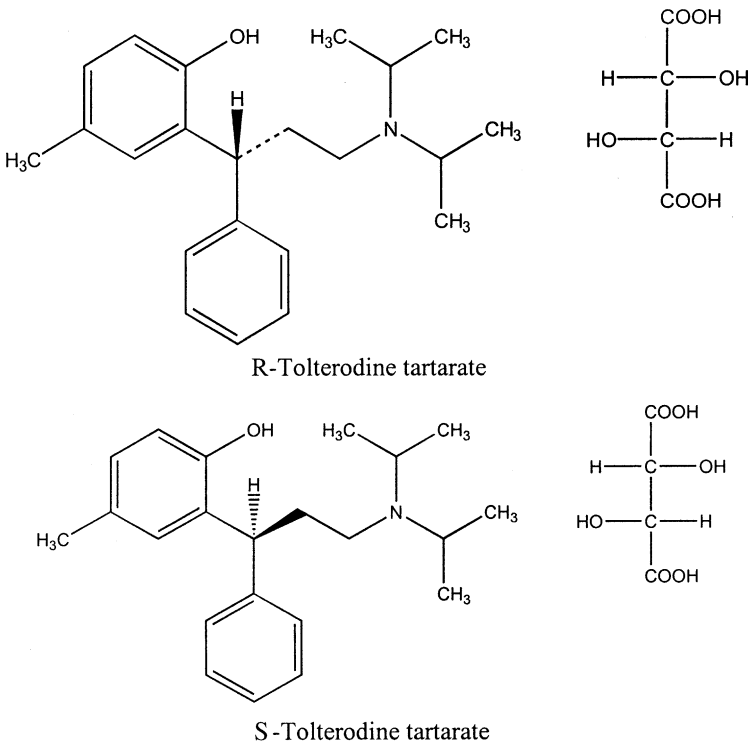

Fig. 1. Structural formula for *R* and *S* tolterodine tartarate.

3.2. Precision

In the precision study the percentage R.S.D. of injection repeatability for (*S*)-isomer was found to be 2.2 and 7.0 at the concentration levels of 2 and 1 μg/ml which is in the acceptable range as per the ICH guidelines. The results are listed in Table 1.

3.3. Quantification of (S)-isomer

Standard addition and recovery experiments were conducted in the presence of large enantiomeric excess of the *R*-isomer to determine accuracy of the present method for the quantification of (*S*)-isomer. The study was carried out at 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0% of target analyte concentration. The correlation coefficient was observed as 0.99994. The results are listed in Table 2. The recovery of (*S*)-isomer was calculated from the slope and the intercept of the calibration curve drawn in the concentrations range of 0.1–0.6%. The percentage recovery was obtained in the range of 98–100%.

Table 1
Precision at the concentration of 2 and 1 μg/ml

Injection no.	*S*-tolterodine area
Precision at the concentration of 2 μg/ml	
1	32551
2	34049
3	32539
4	32694
5	31825
6	32877
Mean	32755
R.S.D. (%)	2.220948
Precision at the concentration of 1 μg/ml	
1	16433
2	16524
3	19502
4	16982
5	18651
6	18275
Mean	17727.83
R.S.D. (%)	7.126858

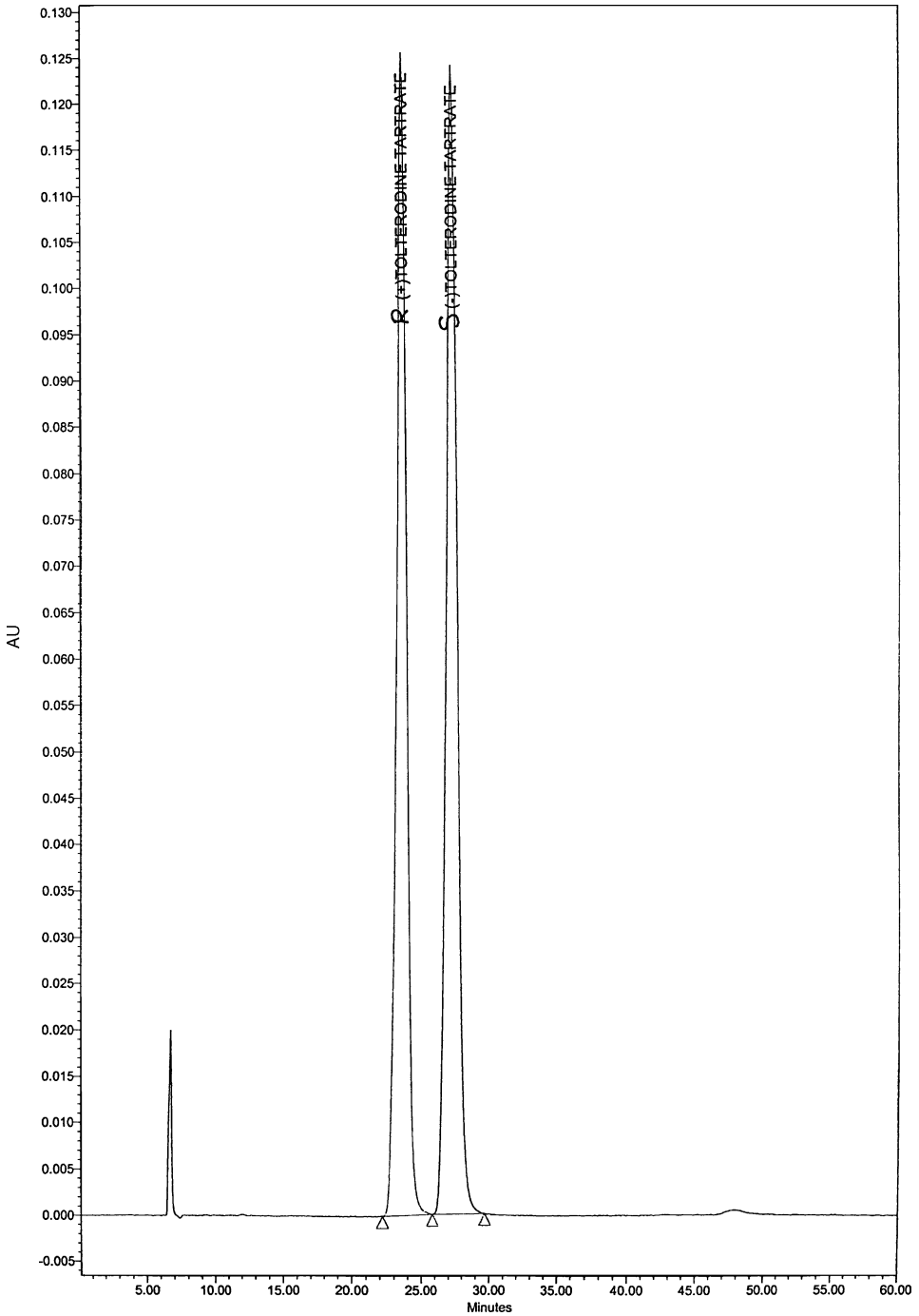

Fig. 2. LC chromarogram of *R* and *S* tolterodine tartarate.

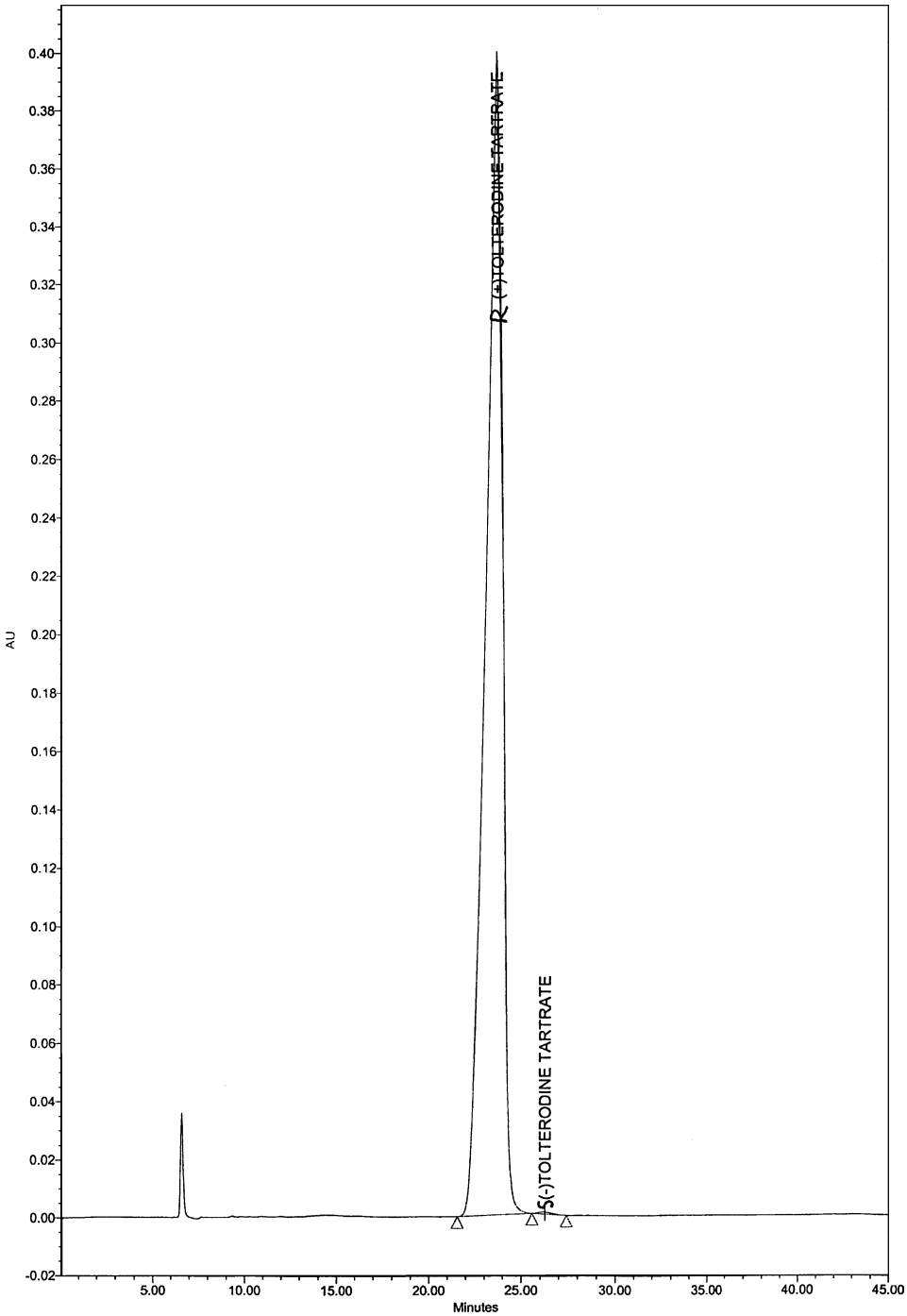

Fig. 3. LC chromarogram of *R* (+) tolterodine tartarate.

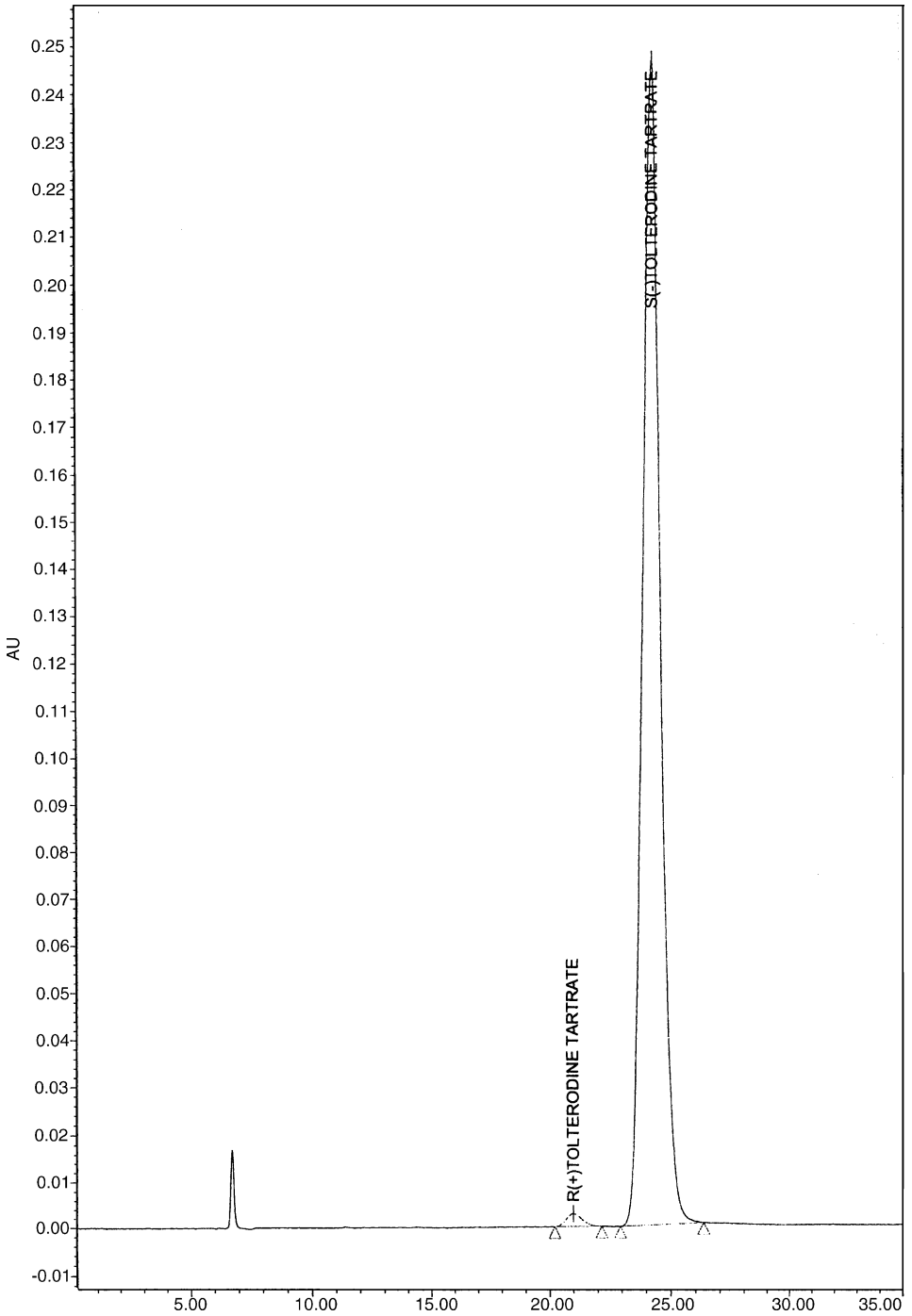

Fig. 4. LC chromarogram of *S* (−) tolterodine tartarate.

Table 2
Quantification of (*S*)-isomer

S. no.	Concentration of *S*-isomer (%)	Area
1	0.05	17425
2	0.10	30869
3	0.25	69701
4	0.50	135746
5	1.0	278893
6	2.0	545485
	Correlation coefficient	0.99994

3.4. Limit of detection and quantification

The limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of 3. LOD for (*S*) isomer was found to be 0.1 μg/ml. The limit of quantification (LOQ) represents the concentration of analyte that would yield a signal to noise ratio of 10. LOQ for (*S*) isomer was found as 0.3 μg/ml.

4. Conclusion

A new chiral LC method was developed for the separation of two isomers of tolterodine tartrate. The method is reproducible and sensitive. The amount of trifluoroacetic acid in mobilephase composition is found to be very crucial in the separation. This method was found to be robust in the estimation of (*S*) isomer up to 0.1 μg/ml.

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