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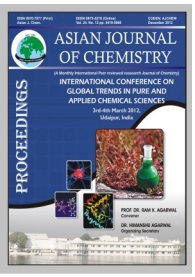

Chiral Separation of Tolterodine Tartrate Using Amylosed Base Immobilized Stationary Phase in LC Method†

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High-performance liquid chromatographic method was developed and validated for chiral separation of tolterodine tartrate. It was achieved on Chiral pack IA (immobilized amylosed based polysaccharides stationary phase) using a mobile phase of hexane: 2-propanol: triethylamine: trifluroacetic acid (91:9:0.2:0.1 v/v) at a flow rate 1.1 mL/min. Resolution between S-tolterodine tartrate and R-tolterodine tartrate was found to be 2.9. Developed LC method was used for the quantification of S-tolterodine tartrate as chiral impurity in R-tolterodine tartrate. The developed method was extensively validated and proved to be robust. The calibration curve for S-tolterodine tartrate showed an excellent linearity over the concentrations range 0.1 to 10 µg/mL. The limit of detection and limit of quantification for S-tolterodine tartrate were 0.11 µg/mL and 0.34 µg/mL respectively. Average recovery of S-isomer was in the range of 97.30 to 101.59 %. S-tolterodine tartrate and R-tolterodine tartrate solutions in mobile phase were found to be stable for 48 h. The proposed method was short runtime, precise and accurate for quantitative determination of S-isomer in R-tolterodine tartrate in API and its solid oral dosage form.

Key Words: S-tolterodine tartrate, Chiral chromatography, Chiral separation, Amylosed based stationary phase, Method validation.

INTRODUCTION

R-Tolterodine tartrate (Fig. 1) is chemically known as (R)-2-[3-[*bis*-(1-methylethyl)-amino]-1-phenylpropyl]-4-methylphenol tartrate[1]. R-tolterodine tartrate is a new muscarinic receptor antagonist intended for the treatment of urinary urge incontinence and other symptoms namely urinary frequency, urinary urgency and incontinence in people with unstable bladders[2]. Tolterodine is a competitive muscarinic receptor antagonist. After oral administration, it metabolized in the liver and resulting in the formation of the 5-hydroxymethyl metabolite, which exhibits an antimuscarinic activity similar to tolterodine and contributes significantly to the therapeutic effect. The main effect of tolterodine is to 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 side of urinary tract[3]. The complete synthesis of tolterodine was also reported[4]. The quantification of S-isomer in R-tolterodine is very important aspect. The literature survey reveals several methods were reported for assay and impurity profiling of tolterodine tartrate[5-10].

Chiral high performance liquid chromatography (HPLC) method for the quantification of S-isomer in R-tolterodine tartrate by using Chiralcel ODH column was reported, but the retention time of both isomers was greater than 25 min and resolution is just adequate *i.e.* 2.5. Sample preparation procedure is quiet complex and author did not given the estimation of S isomer in the solid oral dosage form. The total runtime of analysis of earlier method was *ca.* 1 h, which is too long.

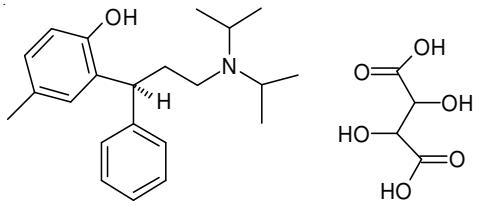

Fig. 1. Structures of R-tolterodine tartrate

To our best of knowledge, there is no short and adequate runtime chiral HPLC method reported for quantification of S-isomer in R-tolterodine tartrate with good resolution. Immobilized amylosed based polysaccharides stationary phase are quite popular with wide recognition for direct resolution of enantiomers due to their variety of organic solvent compatibility and robustness of column. This article mainly deals with quantification of S-isomer in R-tolterodine tartrate using

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normal phase HPLC method. Developed method was validated as per ICH guideline validation parameters such as precision, accuracy, limit of detection, limit of quantification, linearity and robustness[11].

EXPERIMENTAL

S-Tolterodine tartrate and R-tolterodine tartrate were procured from Dr. Reddy's laboratory. Tablet namely detrol was purchased from market. Chiral high performance liquid chromatography grade hexane, 2-propanol was purchased from Rankem Fine Chemicals (India). Trifluroacetic acid and triethylamine was purchased from Merck.

Equipment and chromatographic condition: The HPLC system used was an Agilent Technology (1100 series), equipped with quaternary pump, degasser, auto sampler, column oven and UV detector. The output signal was monitored and processed using Chemstation software. The chromatographic column used was 250 × 4.6 mm ID Chiralpak IA (Daicel Chemical Industries, Ltd., Tokyo, Japan) packed with 5 μm particles. The mobile phase was hexane: 2-propanol: triethylamine: trifluroacetic (91:9:0.2:0.1 v/v). The flow rate of the mobile phase was 1.1 mL/min. The column temperature was maintained at 30 ºC and the UV detector was monitored at a wavelength 284 nm. Injection volume was 20 μL.

System suitability solution and standard solution preparation: S-tolterodine tartrate and R-tolterodine tartrate stock solution (10 mg/mL) was prepared by dissolving appropriate amount of standard into methanol. Stock solution was further diluted with mobile phase to achieve 100 μg/mL concentrations for system suitability solution. S isomer standard solution was prepared to achieve 5 μg/mL concentrations.

Sample preparation: Twenty tablets of mean weight were crushed in mortar. An amount of powdered mass equivalent to 10 mg of R-tolterodine tartrate is transferred to 10 mL volumetric flask. The tablet powder was dissolved and extracted with the methanol. To ensure complete extraction of drug, solution was sonicated for 15 min and diluted to 10 mL with mobile phase. Filtered this solution through 0.45 mm pump nylon filter. This solution contains 1.0 mg/mL concentration of R-tolterodine tartrate.

Method development: The objective of this study was to separate both isomer in short runtime with good resolution. Firstly, we took efforts for the method development on reversed phase chiral HPLC by using series of different stationary phases columns like Chiral AGP, SS Whelk, Chiralpak AD-RH, YMC Chiral NEA and cellulose base stationary phases[12,13]. None of the column shows good resolution between the two isomers of tolterodine tartrate.

Later we tried with normal phase chiral HPLC with immobilized amylosed based polysaccharides stationary phase by using different mobile phase organic solvent composition, acid and base modifiers. Initially we tried by using mobile phase mixture of hexane, ethanol, diethyl amine and trifluroacetic, but we observed only a single peak. Then we replace ethanol with isopropyl alcohol to above mobile phase and we got moderate resolution having tailing of peaks less than 1.5 but the retention of peaks were too long. Later we added triethylamine instead of diethyl amine which makes our work simple by eluting the both isomers peak within 10 min and having resolution 2.5. From this trial it was cleared that the quantity of triethylamine plays an important role in the resolution of isomers. So we tried number of experiment by varying the quantity of triethylamine from 0.1 to 0.3. But we observed that 0.2 % v/v quantity is optimal for good resolution. To improve resolution between two isomers we included methanol in mobile phase and observed only single peak. Various experiments were carried out to select the best mobile phase for optimum resolution and selectivity of two isomers. Excellent separation was achieved on Chiral Pack IA column using mobile phase of hexane: 2-propanol: triethylamine: trifluroacetic (91:9:0.2:0.1 v/v). The system suitability data (Table-1) indicate that the method meets the preliminary requirements and hence ready to be subjected for validation.

TABLE-1
SYSTEM SUITABILITY DATA

Analyte	T_r*	α*	Rs*	N*	T*
S – tolterodine	8.6	-	-	10322	1.3
R – tolterodine	9.8	1.14	2.9	11656	1.2

*T_r -retention time, α -enantioselectivity, Rs -resolution, N -theoretical plates T -tailing factor

Validation of method

Precision: Precision of the method is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple sampling of homogeneous sample. The precision of the method was determined by analyzing six replicate samples of R-tolterodine tartrate tablets (at concentration 1.0 mg/mL).

Linearity: The linearity response of S-tolterodine tartrate was verified at different levels ranging from LOQ to 200 % of the targeted level (5 μg/mL). Seven different concentration solutions containing 0.1-10 μg/mL of S-tolterodine tartrate was prepared. Linearity solutions were injected in triplicate. Calibration curve was obtained by plotting the graph of peak response *versus* concentration data.

Accuracy/recovery: Accuracy of the method was insured by determining recovery of the spiked amount of S-tolterodine tartrate in pre-analyzed sample of R-tolterodine tartrate tablet. The recovery experiment was performed at five levels, 50, 75, 100, 150 and 200 % of the limit.

Limit of detection and Limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) of S-tolterodine tartrate were determined by calibration curve method. Precision at LOD and LOQ was checked by analyzing six replicate injections.

Robustness: To determine the robustness of the method experimental condition were purposely altered. Robustness was evaluated by determining chromatographic resolution. Flow rate was varied by unit ± 0.1 mL/min and column temperature was varied by ± 2 ºC.

Solution stability and mobile phase stability: Stability of S-tolterodine tartrate and R-tolterodine tartrate in solution was studied by keeping the solution in tightly capped volumetric flask at room temperature on laboratory bench for 2 days. Content of S-isomer was checked at 6 h intervals up to 48 h.

RESULTS AND DISCUSSION

The separation of both isomers may be due to interaction between the carbamate group on the stationary phase and hydroxyl group of analyte through hydrogen bonding. These polysaccharides contain a large number of chiral active sites and thus a relative high probability of interaction with the sol ute leading to separation of two isomers[14]. Another possible mechanism is that the π-π interaction between the π-electrons of stationary phase and π-electrons of tolterodine tartrate which are present in phenyl ring. A representative chromatogram of system suitability solution is shown in Fig. 2, showing an excellent resolution (Rs = 2.9) between two isomers and symmetric peak shape with tailing 1.3. The method was found to be precise with % RSD less than 0.8 % for S-tolterodine tartrate content. The response was found to be linear in the range of 0.1 to 10 µg/mL for S-isomer. The calibration curve having correlation of 0.999 was observed with regression equation Y= 8.472x - 0.802. Recovery was found to be in the range of 97.30 to 101.59 % for S-tolterodine tartrate. Recovery data is shown in Table-2. During robustness studies resolution between both isomer was always greater than 2.5, also % RSD for peak area in standard solution was less than 2.0 %. The typical chromatogram test preparation and spike preparation is shown in Figs. 3 and 4 respectively.

Conclusion

A short runtime, simple, specific, linear, precise and accurate normal phase chiral HPLC method has been developed and validated for the separation of tolterodine tartrate isomers. The method was completely validated showing satisfactory data for all validation parameters tested. The developed method can be used for the quantitative determination of S-isomer as chiral impurity in drug substance and its tablet formulation.

TABLE-2
RECOVERY RESULTS OF S-TOLTERODINE TARTRATE (n = 3)

Level (%)	Amount spiked (%)	Amount found (%)	Recovery (%)
0	0	0.11	-
50	0.26	0.36	97.30
75	0.39	0.49	98.11
100	0.52	0.64	101.59
150	0.78	0.88	98.87
200	1.04	1.16	100.87

TABLE-3
VALIDATION RESULTS OF THE DEVELOPED METHOD

Validation parameter	Results
System precision (n = 6, % R.S.D.)	
Peak area	0.9
Retention time	0.2
Method precision (n = 6, % RSD)	0.8
Intermediate precision (n = 6, % RSD)	0.7
Limit of detection (µg/mL)	0.11
Limit of quantification (µg/mL)	0.34
Linearity (0.1 to 10 µg/mL)	
Correlation coefficient	0.999

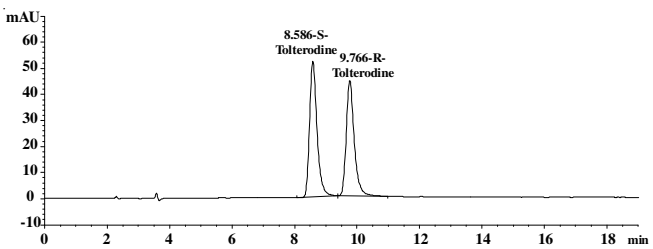

Fig. 2. Chromatogram of resolution solution

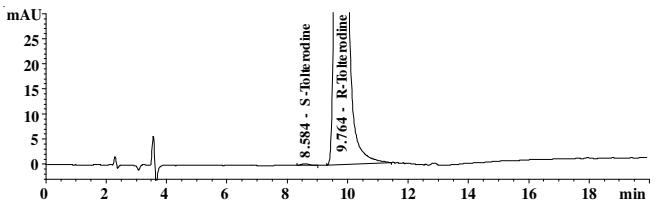

Fig. 3. Chromatogram of R-tolterodine tablet solution

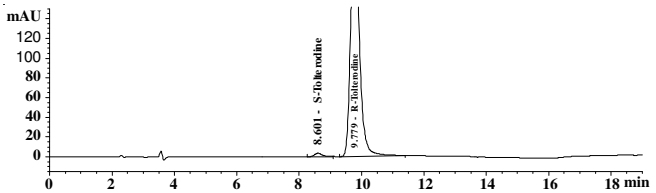

Fig. 4. Chromatogram of 0.5 % spike of S isomer in R-tolterodine tablet solution

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