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A validated LC method for the chiral purity of (S)-1,2,3,4-tetrahydro-1-phenylisoquinoline: A key intermediate of solifenacin

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ABSTRACT

A simple and accurate chiral liquid chromatographic method was described for the chiral purity of (S)-1,2,3,4-tetrahydro-1-phenylisoquinoline [SFN-IV], a key intermediate of Solifenacin in bulk drugs. Separation was achieved with a Chiralpak AD (250 mm × 4.6 mm, 10 µm) column. The ratio of n-hexane, ethanol and triethylamine in the mobile phase were optimized to obtain the best separation. UV detection was performed at 210 nm. The described method is linear over a range of LOQ – 3.75 µg/mL of R-isomer. The mean recovery of R-isomer was found to be in the range of 96–100%. The method is simple, rapid, accurate, selective and precise.

KEYWORDS

Column liquid chromatography;
Enantiomeric separation;
Chiral separation;
Validation and quantification;
Solifenacin;
Key intermediate.

INTRODUCTION

Most of the pharmaceutical industries are now concentrating towards the study of the therapeutic effect of pure enantiomers of the existing drug molecules. A control and accurate quantification of undesired enantiomers in Active Pharmaceutical Ingredient is essential[1] and in this connection use HPLC as monitoring system will be very useful. Solifenacin, marketed as its succinate salt under the trade name *VESIcare*, is a urinary antispasmodic belongs to the anticholinergic class. It is used in the treatment of overactive bladder with or without urge incontinence. It is marketed and manufactured by Astellas and GlaxoSmithKline.

Solifenacin is a single enantiomer and synthesized as (S, R)-enantiomer, since it is pharmacologically more potent than other enantiomers and diasteriomers. SFN-IV is a key starting material in the synthesis of Solifenacin and also chiral in nature. The chiral nature of Solifenacin is due to the presence of chiral moiety of SFN-IV as well as R-Quinuclidinol in the molecule. The content of respective R-isomer present in Solifenacin bulk drug mainly depends on the content of R-isomer present in SFN-IV. Till now a few analytical methods have been reported[2,3] for the determination of Solifenacin in biological samples. To our present knowledge no chiral HPLC methods were reported in the literature for the enantiomeic separation of SFN-IV. Therefore, the aim of this study is to develop a chiral HPLC method for the accurate quantification of un-required isomer [R-isomer] of SFN-IV and validate as per the ICH guideline[4].

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EXPERIMENTAL

Chemicals and reagents

Samples of SFN-IV and R-isomer were synthesized at SMS Pharma Research Centre (Hyderabad, India). The chemical structures of SFN-IV, R-isomer of SFN-IV and Solifenacin were presented in Figure 1. HPLC grade n-hexane and ethanol are obtained from Merck (India). Analytical grade triethylamine is purchased from SD Fine chemicals (India). LC grade water was deionized with Milli-Q Elix and then filtered using Milli-Q gradient, Millipore water purification system (Milford, MA, USA).

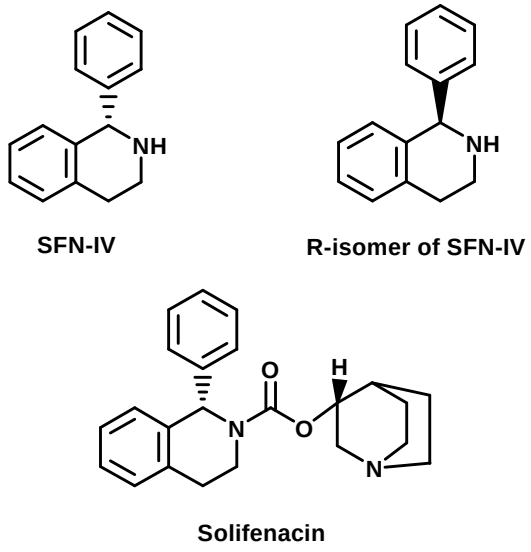

Figure 1 : Structures of SFN-IV, *R*-isomer and solifenacin

Equipment

The HPLC system consisted of quaternary gradient pump, auto sampler, column oven and a variable wavelength detector. The output signal was monitored and integrated using EZ-Chrom Elite Chromatography Data Software (1200 series HPLC, Agilent, USA).

Preparation of standard solutions

Dissolved an accurately weighed quantity of R-isomer working standard in mobile phase to obtain a solution having known concentration of about 0.0025 mg/mL and injected in to the system. Freshly prepared, SFN-IV sample solution (0.25 mg/mL) in mobile phase was injected into the system. As per the approved in-house protocol, different dilutions were made to evaluate specificity, precision, linearity, LOD, LOQ, accuracy and robustness.

Chromatographic conditions

The chromatographic conditions were optimized using an amylose based chiral stationary phase Chiralpak AD (250 mm × 4.6 mm, 10 µm, Daicel make). The mobile phase was n-hexane: ethanol: triethyl amine (95:5:0.1 v/v/v). The flow rate was set at 0.8 mL/min. The detection was carried out at a wavelength of 210 nm. The injection volume was 10 µL. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

RESULTS AND DISCUSSION

Method development

The aim of this work is to separate the enantiomers of SFN-IV and accurate quantification of R-isomer.

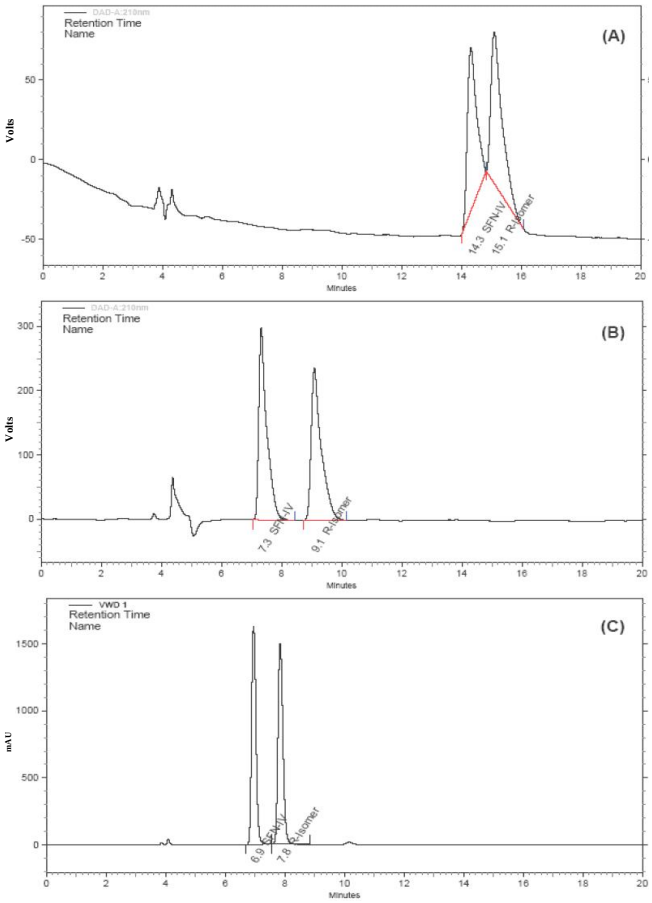

Figure 2 : Typical chromatogram of enantiomeric separation of racemic SFN-IV on (A) Chiralcel OJ-H, (B) Chiralcel OD and (C) Chiralpak AD columns

The racemic mixture was prepared by physical mixing the equal portions of SFN-IV and R-isomer (0.1 g of each sample). A 0.25 mg/mL solution of racemic mixture prepared in mobile phase was used during the method development. To develop a rugged and suitable LC method for the enantiomeic separation of SFN-IV, different mobile phases and stationary phases were employed. Three different chiral columns were employed during method development namely Chiralcel OJ-H, Chiralcel OD, and Chiralpak AD of Daicel. The mechanism of separation in direct chiral separation methods is the interaction of chiral stationary phase (CSP) with analyte enantiomers to form a short lived, transient diastreomeric complexes. Various experiments were conducted, to select the best stationary and mobile phase that would give optimum resolution and selectivity for the enantiomers. The enantiomeric separation of SFN-IV on Chiralcel OJ-H, Chiralcel OD and Chiralpak AD columns is shown in Figure 2.

Optimized chromatographic condition

Better baseline resolution achieved on Chiralpak AD column, using a mobile phase system containing n-hexane: ethanol: triethyl amine (95:5:0.1, v/v/v). In the present method the selectivity was found to be more than 1.1 with a resolution greater than 3.0 between SFN-IV and R-isomer.

Validation of the method

The LC method developed has been extensively validated for the quantitation of R-isomer in SFN-IV using the following parameters. Standard solution was used for the purpose of quantification of R-isomer in SFN-IV.

Specificity

SFN-IV and R-isomer were injected separately to confirm the retention times. System suitability solution was then injected. SFN-IV and R-isomer peaks are eluted at 6.9 and 7.8 minutes respectively (relative retention 1.13). The resolution between the peaks was found to be 3.2. The asymmetry was found to be 1.2 for both R-isomer and SFN-IV peaks.

Precision

Repeatability was demonstrated by analyzing SFN-IV sample 6 times. Intermediate precision was demonstrated by analyzing same sample of SFN-IV by two different analysts on two different days. Intra-day variations of R-isomer content in SFN-IV are expressed in terms of %RSD values. The values calculated were found to be 1.2 for repeatability, 1.5 and 1.1% for intermediate precision. Repeatability and intermediate precision results are shown in TABLE 1.

TABLE 1 : Precision results for *R*-isomer

Repeatability	
Mean of *R*-isomer content (%, *m/m*) *(n=6)*	0.424
Standard deviation (SD)	0.0049
%RSD	1.2
Intermediate Precision	
Analyst-1/Day-1	
Mean of *R*-isomer content (%, *m/m*) *(n=6)*	0.424
Standard deviation (SD)	0.0049
%RSD	1.2
Analyst-2	
Mean of *R*-isomer content (%, *m/m*) *(n=6)*	0.427
Standard deviation (SD)	0.0062
%RSD	1.5
Overall %RSD *(n=12)*	1.3
Day-2	
Mean of *R*-isomer content (%, *m/m*) *(n=6)*	0.421
Standard deviation (SD)	0.0045
%RSD	1.1
Overall %RSD *(n=12)*	1.2

Limit of detection and limit of quantification

The limit of detection and limit of quantification for R-isomer was calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve. A typical S/N ratio of 2-3 and 9-10 are generally considered to be acceptable for LOD and LOQ respectively. LOD and LOQ values are found to be 0.0160µg/mL and 0.0485µg/mL respectively.

Linearity

Standard solutions at ten different concentration levels ranging from 0.125, 0.250, 0.500, 0.750, 1.000, 1.250, 1.875, 2.500, 3.125 and 3.750µg /mL were prepared (0.05 – 1.5% of analyte concentration of 0.25mg/mL). Each sample solution was injected in triplicate. The mean responses recorded were plotted against concentration. The correlation coefficient for R-

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isomer was found to be 0.9999, which indicated good linearity. The calibration equation for R-isomer was found to be y = 86123x – 1347.

Accuracy

SFN-IV sample was spiked with R-isomer at 0.5, 1.0 and 1.5% of analyte concentration of 0.25mg/mL. Each spiked solution was prepared in triplicate and injected. The mean recoveries, recovery percentage and %RSD were calculated. The mean recoveries of R-isomer at each spike solution with 95% confidence level are found to be 95.9±0.42%, 100.0±0.31% and 98.6±0.57% respectively. The acceptance criteria for recovery at each level is between 80 and 120% as per in-house validation protocol. Accuracy results are shown in TABLE 2.

TABLE 2 : Accuracy results for *R*-isomer

R-isomer spike level (%, *m/m*)	Added (µg) (n=3)	Recovered (µg)	% Recovery	Mean Recovery	%RSD
0.5	62.95	60.33	95.8	95.9	0.4
		60.60	96.3		
		60.14	95.5		
1.0	125.9	126.22	100.3	100.0	0.3
		125.56	99.7		
		126.02	100.1		
1.5	188.85	186.95	99.0	98.6	0.5
		185.20	98.1		
		186.71	98.9		

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ± 0.1 mL/ min and change in the ratio of mobile phase (± 30% relative). The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.

CONCLUSION

The present paper describes the development of a new chiral HPLC method for the determination of chiral purity of SFN-IV a key intermediate for Solifenacin and its validation. The method was found to be selective, sensitive, precise and accurate for the quantitation of R-isomer. This method can be used for the routine analysis in pharmaceutical quality control.

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