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Short communication

Enantiomeric separation of a moxifloxacin intermediate by chiral liquid chromatography using cellulose based stationary phases☆

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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 chromatographic 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 [1–3]. Several CSPs are now available to allow the direct separation and determination of drug enantiomers and racemates. Cellulose CSPs are one of these commonly employed phases used for the separation and enantiomeric purity determination. The ability of chemically modified cellulose to separate a variety of racemates has recently been reviewed by Okamoto [4]. Enantiomeric inclusion in chiral cavities, which might be multiple, and competitive in cellulose and amylose based CSPs seems to be responsible for the chiral discrimination [5]. Especially if modified as tris carbamates or Tri esters, the corresponding CSPs exhibit excellent resolution properties.

Fluoroquinolones are widely used as antimicrobial agents against Gram-negative and Gram-positive bacteria. Moxifloacin is a new 8-methoxy quinoline drug and discovered by Bayer. As compared to ciprofloxacin and sparfloxacin, moxifloxacin is reported to possess a significantly better antibacterial activity especially against Gram-positive bacteria [6].

Moxifloxacin (**I**) was synthasised by condensation of (S,S)-2,8-diazabicyclo(4.3.0) nonane (**II**) with 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid(**III**). **II** was prepared by debenzylation of optically pure

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(S,S)-8-benzyl-2,8-diazobicyclo(4.3.0) nonane (**V**). **V** was obtained by optical resolution of (±)-*cis* 8-benzyl-2,8-diazobicyclo(4.3.0) nonane (**IV**) by separation of *cis*-(R,R)-isomer(VI) as crystalline L-(+)-tartrate and further purification of the *cis*-(S,S)-isomer (**V**) as the D-(−)-tartrate [3]. All the structures of the above compounds are shown in Fig. 1. The enantiomeric purity of **V** is very important for the synthesis of moxifloxacin. Hence it is necessary to develop an elegant analytical method, till date not available in literature, to determine the chiral purity of **V**. This paper describes the methods involving the CSPs such as chiralcel OD-H, chiralcel OJ and chiralcel OB columns. Chiralcel OD-H column is having a cellulose carbamate derivative as CSP while both chiralcel OJ and OB are having cellulose ester derivatives as their CSPs. The effect of the organic modifiers, 2-propanol and ethanol in the mobile phase was also studied. The best of the three methods has been validated.

2. Experimental

2.1. Equipment

A LC solvent system (Waters 510 pump, Millipore) equipped with an injector (Rheodyne 7125NS-005), a variable UV-Visible detector (Waters 486), Millenium software (Waters Chromatographic Manager 2010) was used. A 15 cm × 4.6 mm I.D., 5 μm chiralcel OD-H (Daicel, Japan) column was used in the developed method. The other CSP columns investigated in this experiment were a 25 cm × 4.6 mm I.D., 10 μm chiralcel OJ with a 5 cm guard column of No. OJ00CC-HK016 (Daicel), a 25 cm × 4.6 mm I.D., 10 μm chiralcel OB with a 5 cm guard column of No. OB00CC-GJ008 (Daicel), a 25 cm × 4.6 mm I.D., 10 μm Chiralcel OD-R (Daicel), a 25 cm × 4.6 mm I.D., 5 μm crownpack CR (−) (Daicel) and a 25 cm × 4.6 mm I.D., 5 μm cyclobond I 2000 Ac (Astec, USA).

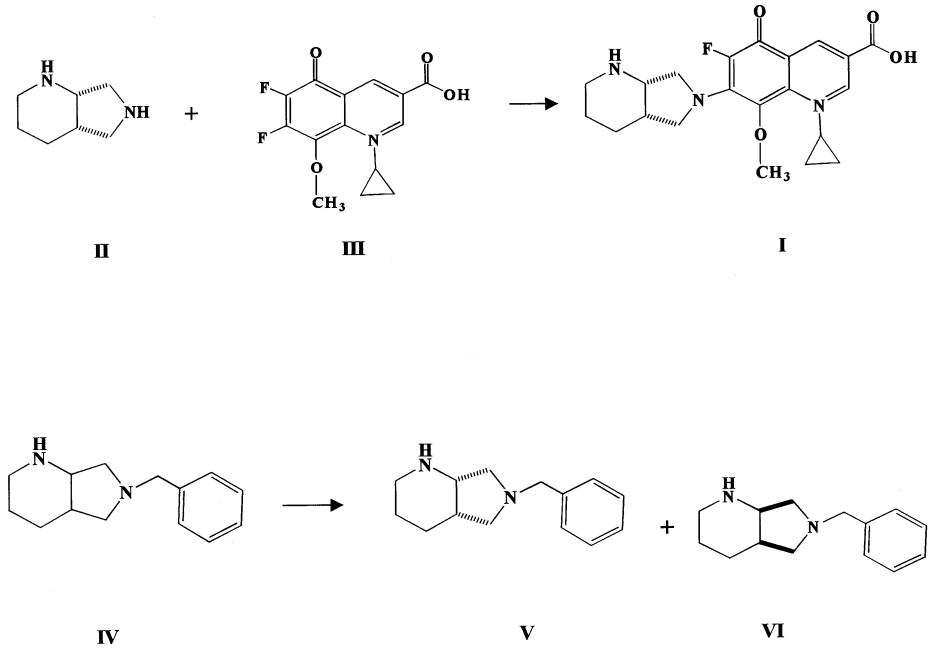

Fig. 1. Structures of moxifloxacin and its intermediates.

2.2. Materials

HPLC- grade hexane, 2-propanol and ethanol were procured from Merck (E-Merck India). Diethylamine was purchased from Spectrochem (Spectrochem Pvt., India). The racemic mixture, (S,S)-isomer and (R,R)-isomer was obtained from Process Research and Development of Dr Reddy's Research Foundation (Hyderabad, India).

2.3. Preparation of the sample solutions

Samples of (S,S)-isomer, (R,R)-isomers and the racemic mixture were dissolved in minimum amount of 2-propanol(or ethanol) and made up with mobile phase to a concentration of 25 mg/ml. These solutions were further diluted with mobile phase to obtain a concentration of 10.0 mg/ml and were used for the study.

2.4. Chromatographic conditions

The chromatographic separations were performed using a Chiralcel OD-H, 5 μm, 150 × 4.6 mm column and using a mobile phase, Hexane: 2-propanol: diethylamine (99.4:0.5:0.1, v/v/v); the flow-rate of the mobile phase was 1.0 ml/min; the samples were monitored with a UV detection at 230 nm; a 10 μl volume of the sample was injected into the HPLC system.

3. Results and discussion

Various preliminary trials were conducted in the reversed phase (RP) mode to select the stationary and mobile phases that would give an optimum separation and selectivity for the (R,R) and (S,S) enantiomers using chiralcel OD-R (Daicel make) and cyclobond I 2000 Ac (astec make) and crownpack CR (−) (Daicel make) column. There was no indication that separation is possible with these columns. Then normal-phase mode (np) was employed using the chiralcel OD-H, OJ and OB Columns with different combinations of hexane, ethanol and 2-propanol. Initially here also no indication for separation of the enantiomers was observed. However, after the addition of small quantity of diethylamine (0.1%) to a mobile phase containing hexane and 2-propanol (95:5, v/v), a limited separation of the enantiomers on all three columns was observed. The presence of diethylamine (DEA) in the mobile phases plays an important role in the retention of the two enantiomers and also in improving the chromatographic efficiency. Various experiments were conducted on the combinations of hexane, 2-propanol and diethylamine to obtain a mobile phase that would give optimum resolution and selectivity. Surprisingly, a good resolution and selectivity were obtained using the same mobile phase hexane:2-propanol:DEA (99.4:0.5:0.1, v/v/v) on chiralcel OD-H and OB columns. Adequate resolution and selectivity were observed with a mobile phase consisting of hexane: 2-propanol: DEA (99.0:1.0:0.1, v/v/v) on the chiralcel OJ column. Chromatograms of *cis*-racemic sample using chiralcel OD-H, OJ and OB columns are shown in Fig. 2. This indicates that those chiralcel OD-H, OJ and OB stationary phases showing almost the same selectivity towards these enantiomers. Capacity factors, resolution and selectivity of (S,S)-isomer and (R,R)-isomer on the three columns are given in Table 1. In all these experiments the undesirable (R,R)-isomer eluted prior to the required (S,S)-isomer. This facilitates accurate determination of enantiomeric excess [7] of (S,S)-enantiomer.

The selectivity and resolution of the two enantiomers varies with the choice of the alcohol. Hence, use of ethanol instead of 2-propanol was studied, applying the above-mentioned conditions on the three columns. Early elution with limited resolution was observed on chiralcel OD-H and OJ columns, but on chiralcel OB with ethanol instead of 2-propanol, the resolution and peak symmetry was significantly improved. Chromatograms of a *cis*-racemic sample using chiralcel OD-H, OJ and OB columns analysed in ethanol system are shown in Fig. 3. The results of system suitability tests are presented in the Table 1. In ethanol systems also (R,R)-isomer comes earlier to its mirror image on all the three columns.

Finally we conclude from the results presented in Table 1, that Chiralcel OD-H with the mobile phase consisting of hexane:2-propanol:DEA

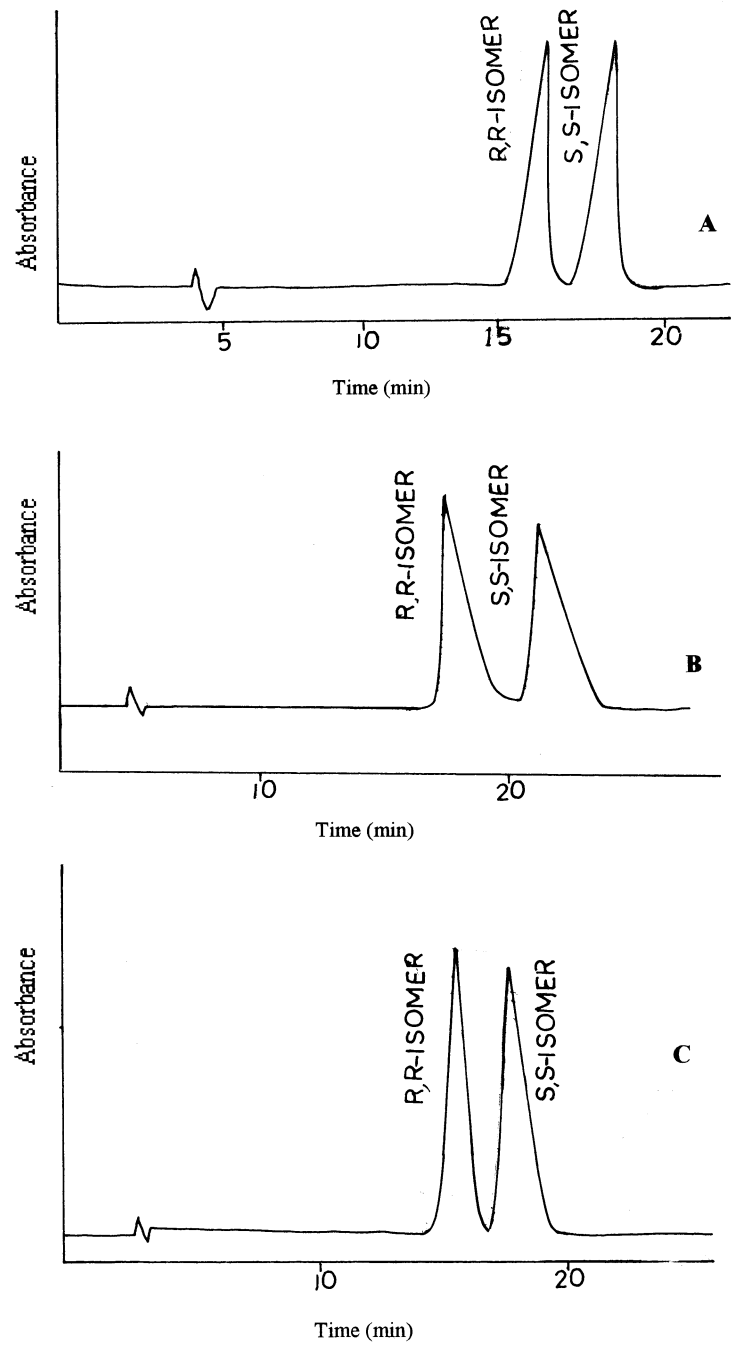

Fig. 2. Chromatograms of *cis*-recemic (**IV**) sample (10 mg/ml) using 2-propanol as organic modifier in the mobile phase; A, on chiralcel OD-H column; B, on chiralcel OB column; C, on chiralcel OJ column.

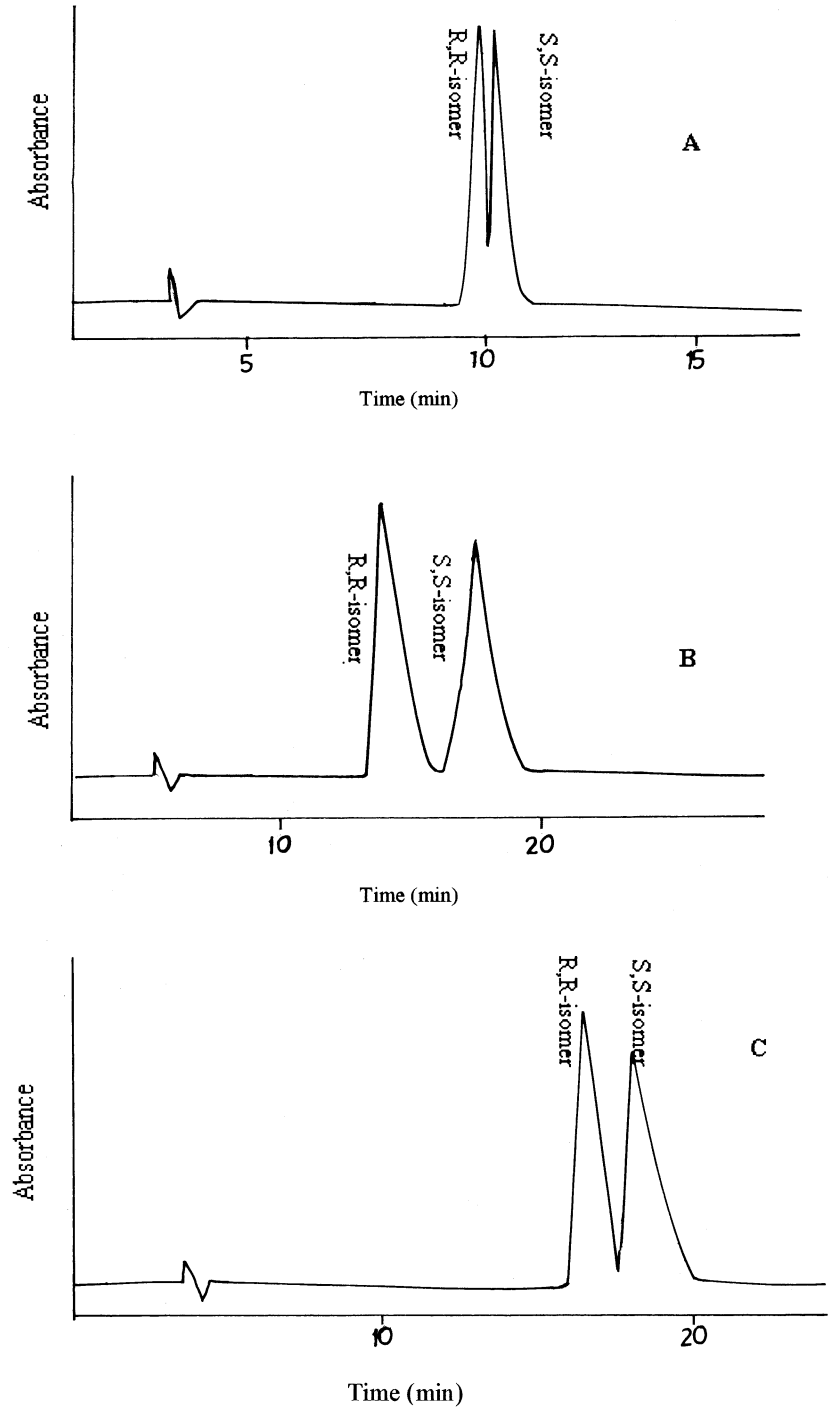

Fig. 3. Chromatograms of *cis*-recemic (**IV**) sample (10 mg/ml) using ethanol as organic modifier in the mobile phase; A, on chiralcel OD-H column; B, on chiralcel OB column; C, on chiralcel OJ column.

Table 1
System suitability results of three columns using 2-propanol or ethanol as organic modifier

Column	Capacity factor[a]		Resolution[a]	Selectivity[a]
	Cis-RR	*Cis*-SS		
Chiralcel OD-H	15.1 (9.55)[b]	16.9 (10.12)	2.2 (0.8)	1.20 (1.05)
Chiralcel OB	17.4 (12.4)	22.2 (16.6)	1.6 (1.8)	1.27 (1.30)
Chiralcel OJ	15.6 (15.6)	18.4 (17.38)	1.6 (1.2)	1.18 (1.16)

[a] Number of samples analysed is 3.
[b] The values entered in brackets belong to ethanol system.

(99.4:0.5:0.1, v/v/v) system is the best of all the systems investigated. The remaining two columns, chiralcel OB with a mobile phase of hexane:ethanol:DEA (99.4:0.5:0.1, v/v/v) and chiralcel OJ, a mobile phase of hexane:2-propanol: DEA(99.4:0.5:0.1, v/v/v), showed adequate resolution and selectivity. Hence, these systems may be used as alternate systems for the separation of the (S,S)-isomer and (R,R)-enantiomer. The method using the chiralcel OD-H column was validated by conducting standard addition and recovery of (R,R)-isomer in (S,S)-isomer. The detection limit and quantitation limit for (R,R) isomer were found to be 2.5 and 8 μg/ml, respectively for 10 μl volume of injection.

At the end of every day, the column (chiralcel OD-H) was washed with ethanol with a flow rate of 0.5 ml/min for 1 h and stored in hexane:2-propanol (90:10) mixture overnight.

3.1. Precision and recovery

Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of traces of the (R,R)-isomer present in the (S,S)-isomer.

The range of addition levels used in this study was 0.2–1.0%. Recovery of (R,R)-isomer added to (S,S)-enantiomer can be calculated from the slope and γ-intercept of the calibration graph of (R,R)-isomer drawn in the range of concentrations 10–150 μg/ml (0.1%–1.5% of analytical concentration of 10.0 mg/ml). The slope and intercept were 932.38 and 3215.9, respectively, with a correlation coefficient 0.992. Recovery of the (R,R)-isomer was with an average of 98.8, with an RSD below 2.0%. The precision of the method was determined by analysing six independent samples by two analysts on the same day on two columns, using different instruments and mobile phase preparations. The precision of the method is found to be 3.0% (RSD) (Table 2).

The possibility of potential chiral-inversion of *cis*-(S,S)-enantiomer in the sample preparation was also investigated. For three independent samples of *cis*-(S,S)-enantiomer, 0.3% of (R,R)-isomer was added to each sample. They were analysed for three successive days. After each day analysis samples were stored at RT under darkness. From the results obtained (RSD $< 2.5\%$), it is clear that *cis*-(S,S)-isomer is chirally stable in solution for at least 3 days.

4. Conclusion

A np chiral LC method was described for the separation of two enantiomers of (±)-*cis* 8-ben-

Table 2
Precision for the analysis of *cis*-RR isomer present in *cis*-SS enantiomer

Analyst	Sample preparation	Recovery of *cis*-RR isomer (μg)
I	1	40.8
	2	41.7
	3	41.5
II	1	43.4
	2	44.2
	3	42.3
		S.D.: 1.27 R.S.D.: 3.0%

zyl-2,8-diazobicyclo(4.3.0) nonane, an intermediate of Moxifloxacin using chiralcel OD-H column. The method is simple, reproducible and sensitive. The separation of two enantiomers was also obtained on chiralcel OJ and OB columns with adequate resolution and selectivity. The developed methods are useful for the analysis of the in-process samples.

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