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Journal of Pharmaceutical and Biomedical Analysis 33 (2003) 803–809
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Enantiomeric separation of the key intermediate of paroxetine using chiral chromatography

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Received 6 December 2002; received in revised form 10 April 2003; accepted 20 May 2003

Abstract

A selective and reproducible chiral LC method has been developed for the separation and quantification of a key intermediate of paroxetine. The separation was achieved on three different chiral stationary phases, viz Chiralcel OD (250 × 4.6 mm, 10 μm), Chiralpak AD (250 × 4.6 mm, 10 μm) and Chiralcel OJ (250 × 4.6 mm, 10 μm). The method was validated on the Chiralcel OD phase using a mobile phase system consisting of hexane, isopropanol, and diethylamine in the ratio of 96:04:0.3 v/v/v. The precision (% R.S.D.) of the method was found to be less than 1.0 with the percentage recoveries of II B ranged from 96.0 to 103.4. The limits of detection and quantification of II B were found to be 2.0 and 7.5 μg/ml, respectively. The method was linear, with a correlation coefficient greater than 0.990, and the method was proved to be rugged.

Keywords: Paroxetine; Chiral LC; Validation; Correlation coefficient

1. Introduction

Paroxetine (III) is an antidepressant, contains phenyl piperidine moiety, which acts by specially inhibiting the reuptake of 5-hydroxytryptamine into brain neurons. The trial results show an elevation of mood in patients usually within 14 days from start of treatment as well as an improvement of the Hamilton depression rating scale and the clinical global impression scale. In panic disorder, paroxetine is as active as clomipramine, may be faster acting and better tolerated, and is effective for at least up to 1 year. Side effects have found to be very less in the clinical trials [1,2].

Most of the synthetic routes developed towards the manufacturing of active pharmaceutical ingredient (API) of paroxetine involve the key intermediate racemic *trans* (3RS, 4SR)-1-benzyl-4-(4-fluorophenyl)-3-hydroxymethylpiperidine (I). As the API paroxetine has the stereochemistry of 3S

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0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00313-3

and 4R in the phenyl piperidine moiety and the racemic key intermediate (I) need to be resolved. This resolution is done using the chiral reagent di p-toluoyl D-tartaric acid to obtain the required isomer (−) *trans* (3S, 4R)-1-benzyl-4- (4-fluorophenyl)-3-hydroxymethylpiperidine (II A). This is controlling step, as a very high enantiomeric purity of final API is required, and during this process, (+) *trans* (3R, 4S)-1-benzyl-4-(4-fluorophenyl)-3-hydroxymethylpiperidine (II B) is carried as the impurity. (−) *trans* (3S, 4R)-1-benzyl-4-(4-fluorophenyl)-3-hydroxymethylpiperidine (II A) is condensed with 3,4-methylenedioxy phenol and then debenzylated to get paroxetine (III). The schematic diagram describing these steps for paroxetine is given in Fig. 1. In the literature no chiral LC methods have been reported for the quantitative determination of the paroxetine key intermediate and its enantiomer.

2. Experimental

2.1. Materials

HPLC grade hexane (fraction from petroleum) and isopropanol were purchased from S.D.finechem (India), ethanol from Les alcools De Commerce Inc (Ontario), diethylamine from Spectrochem (India), trifluoroacetic acid from Lancaster (England), samples of II A and II B were obtained from Technology Development Centre of Dr Reddy's Laboratories Limited (Hyderabad, India).

2.2. Equipment

The LC system used in laboratory consisted of a Waters Alliance 2690 solvent delivery system, auto injector, and a waters 996 photo diode array detector. The output signal was monitored and

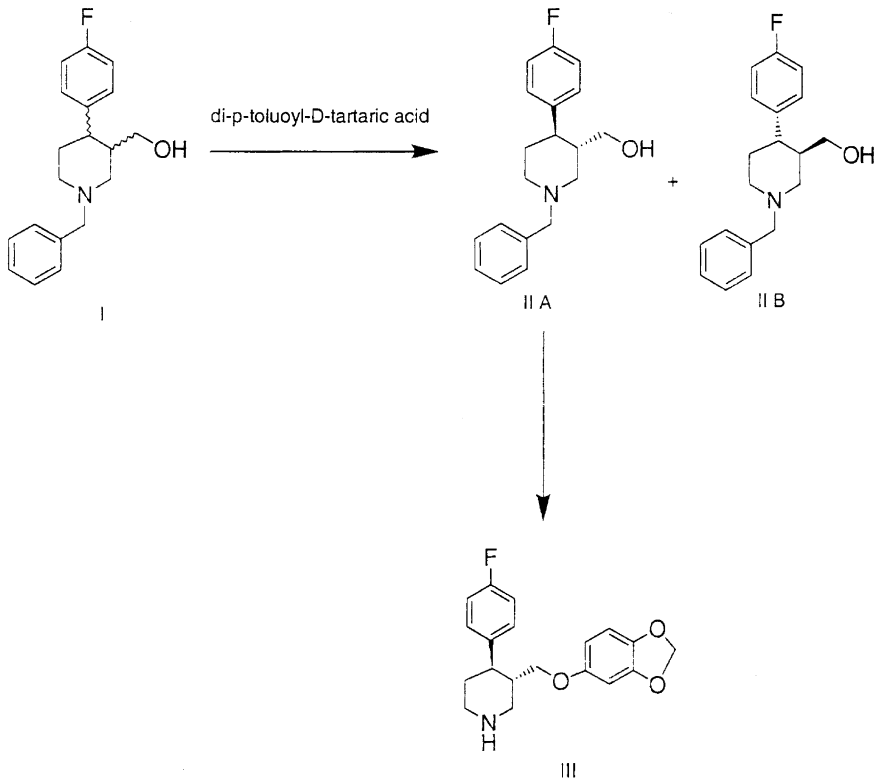

Fig. 1. Chemical structures of paroxetine (III), (−) *trans* isomer (II A) and racemic (I).

processed using a MILLENNIUM 2010 CHROMATOGRAPHY MANAGER software version 2.15 (Waters) on pentium computer (Digital Equipment Co). HPLC columns used were Chiralcel OD, Chiralpak AD, Chiralcel OJ and Chiralcel OB all from Daicel Chemical Industries, Tokyo, Japan, having dimensions of 250 mm length, 4.6 mm ID and 10 μm particle size.

2.3. Chromatographic conditions

The mobile phase system consisted of hexane, 2-propanol and diethylamine in the ratio of (96:04:0.3 v/v/v). The flow rate was 1.0 ml/min. The column temperature was maintained at 25 °C and the eluent was monitored at a wavelength of 265 nm. The injection volume was kept at 10 μl. The retention times of II A and II B were around 14 and 18 min, respectively.

2.4. Sample preparation

The stock solutions of II A and II B were prepared by dissolving in isopropanol and making up the solution with the mobile phase to yield a concentration of 10.0 and 0.5 mg/ml, respectively. These stock solutions were further diluted with mobile phase to the desired concentrations.

3. Results and discussion

3.1. Method development

Normal phase columns such as Chiralpak AD, Chiralcel OD and Chiralcel OJ were selected to develop a suitable method. Using diethylamine (0.3%) in a hexane, isopropanol mixture (96:4), good resolution was achieved between II A and II B on a Chiralcel OD (250 × 4.6 mm, 10.0 μm) column. A typical chromatogram is shown in Fig. 2C.

Excellent separation was also achieved using Chiralpak AD (250 × 4.6 mm, 10.0 μm) with a mobile phase consisting of hexane, ethanol and trifluoroacetic acid in the ratio 93:07:0.3 v/v/v, as shown in Fig. 2B. In both cases II A eluted prior to II B. It is assumed that chiral discrimination on these CSPs was due to the formation of solute–CSP complexes through inclusion of the enantiomers in to the chiral cavities in the higher order structures of the chiral stationary phases [3–5]. In the case of CSPs with carbamate derivatives, the binding of the solutes to the CSPs was achieved through interactions between the solutes and the polar carbamate groups on the CSPs [6,7]. The carbamate groups on the CSPs can interact with solutes through hydrogen bonding using the C=O and NH groups, and through dipole–dipole interaction using the C=O moiety. In our study the available functional group on the solute is –OH, which can form hydrogen bonding with the C=O group on the CSPs. Wainer et al. [6] have reported that solutes having aromatic functionalities could provide additional stabilising effect into the solute–CSP complex by insertion of the aromatic portion of the solute into the chiral cavity. In our case, this type of stabilisation effect may also exist due to the presence of the aromatic functionality on the solute.

With Chiralcel OJ and a mobile phase consisting of hexane:ethanol:trifluoroacetic acid (93:07:0.3 v/v/v) good separation was observed with a reversed elution order. A typical chromatogram using the Chiralcel OJ column is shown in Fig. 2A. The enantiomeric elution order was reversed and this shows that diastereomeric complexes between the CSP and the II A may be more stabilised with less bulky alcohols such as ethanol as the mobile phase modifier. Enantiomers were co-eluted when isopropanol was used in place of ethanol. This phenomenon can be explained by the difference in the steric bulkiness around the hydroxyl moiety of the mobile phase modifier. The less bulky alcohols could be inserted in to the cavity of the CSP more easily than the bulkier alcohols. The insertion of the mobile phase modifier into chiral cavities of the CSP could induce changes in the dominant chiral recognition mechanism leading eventually to inversion of the enantiomeric elution order [8]. The elution order using Chiralcel OJ, Chiralpak AD and Chiralcel OD columns are shown in Fig. 3D–F, respectively.

The Chiralcel OD column was selected for validation because of its higher selectivity compared with the other two columns. System suit-

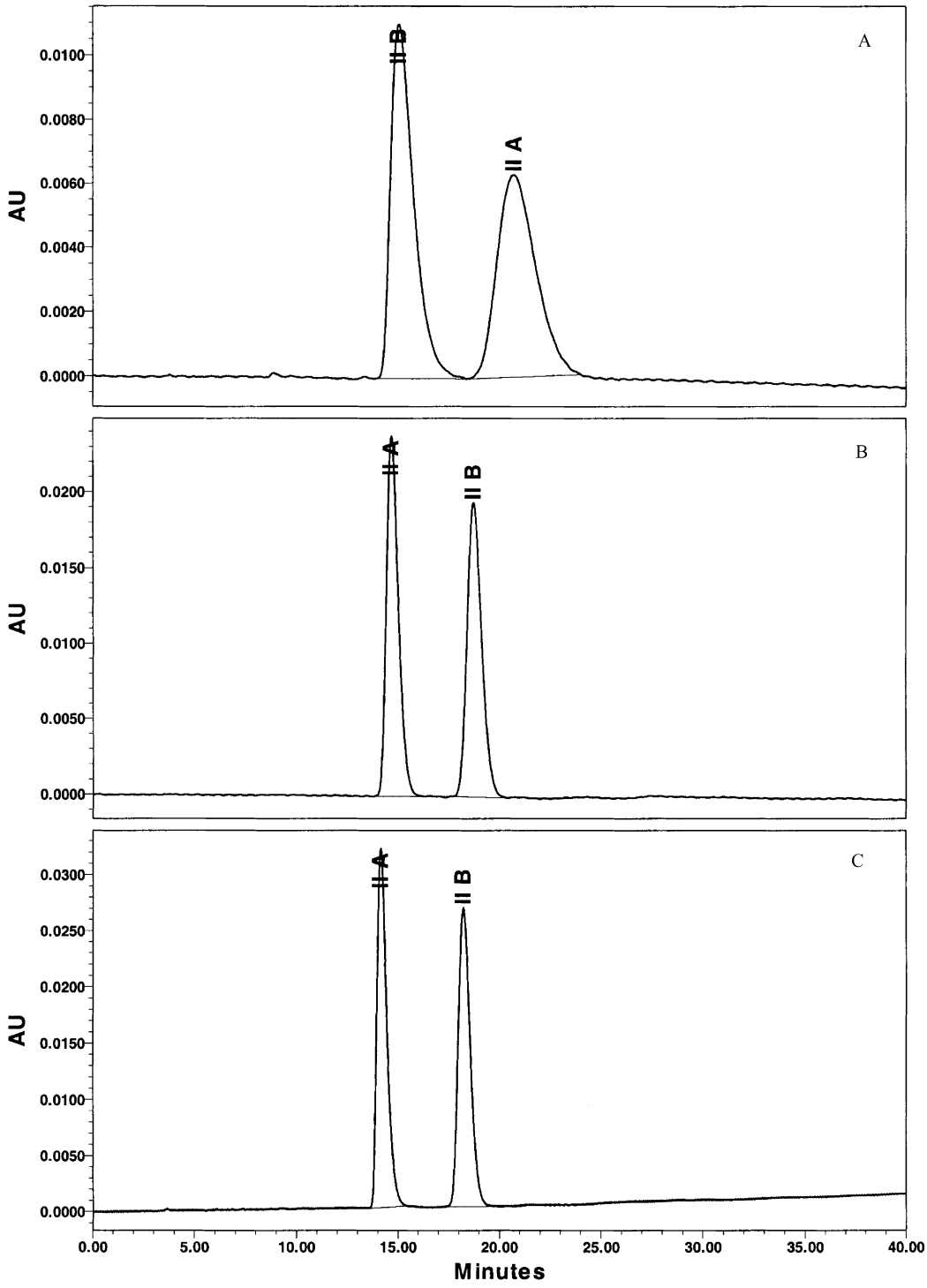

Fig. 2. HPLC chromatogram of (±) *trans* isomer using three different columns. (A) On Chiralcel OJ column. (B) On Chiralpak AD column. (C) On Chiralcel OD column.

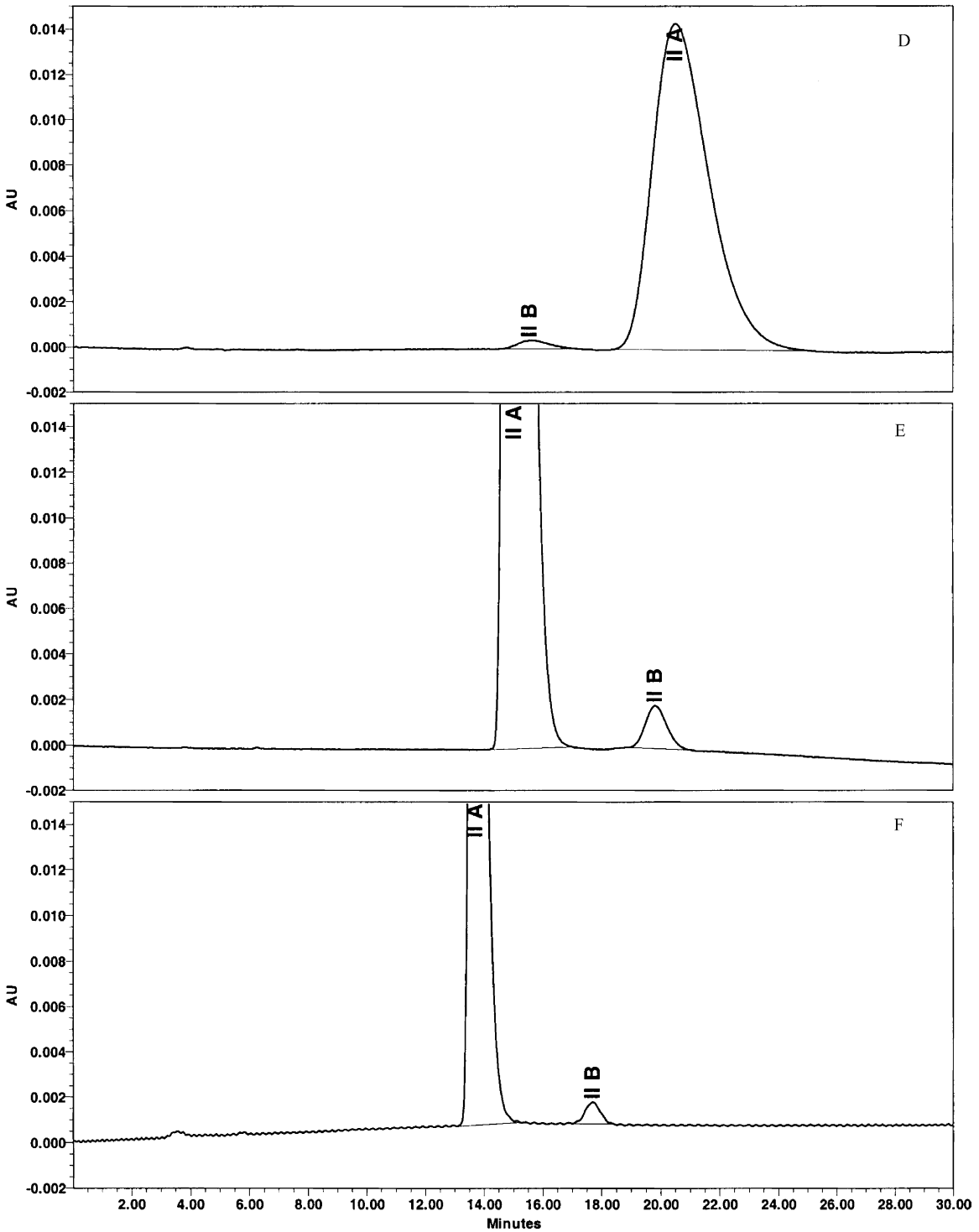

Fig. 3. HPLC chromatogram of (−)*trans* and (+) isomer elution order in three different columns. (E) On Chiralcel OD column. (D) On Chiralcel OJ column. (F) On Chiralpak AD column.

Table 1
System-suitablity report

Column	HPLC conditions	Rs	T	α	k_1'	k_2'
ChiralcelOJ (250 × 4.6 mm) 10 μm	Hexane:ethanol:TFA, 93:07:0.3 v/v/v, flow: 1.0 ml/min, UV: 265 nm	2.0	1.7	1.4	19.8	14.1
ChiralpakAD (250 × 4.6 mm) 10 μm	Hexane:ethanol:TFA, 93:07:0.3 v/v/v, flow: 1.0 ml/min, UV: 265 nm	3.2	1.3	1.2	13.7	17.7
Chiralcel OD (250 × 4.6 mm) 10 μm	Hexane:isopropanol:DEA, 96:04:0.3 v/v/v, flow: 1.0 ml/min, UV: 265 nm	3.6	1.2	1.3	13.2	17.2

Rs, USP resolution; T, USP tailing; α, Selectivity; k′, capacity factor. k_1', capacity factor for II A. k_2', capacity factor for II B.

ability results of the above mentioned three columns are given in Table 1.

3.2. Method validation

3.2.1. Linearity

The linearity of the method was checked by preparing solutions at concentrations of 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0% for II B and 50, 75, 100, 125 and 150% for II A. The linearity of the method for both II A and II B was studied and the data were subjected to linear regression analysis. The calibration curves in both cases showed good linearity with a correlation coefficient (r2) greater than 0.990. The calibration equation for II B was $y = 1325.762x + 260.9078$, while it was $y = 76517.06x + 16172.16$ for II A.

3.2.2. Accuracy and precision

Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of traces of the II B present in the II A.

II B was spiked with II A at different concentrations of 0.8, 1.0, and 1.2% of analyte concentration 1.0 mg/ml (in triplicate). The recovery of II B ranged from 96.0 to 103.0% with an average of 100.1% (Table 2).

Precision solution was determined by spiking II B with the II A at a concentration of 1.0% of the target analyte concentration and this was analysed ten times. The precision of the method was found to be less than 1.0% R.S.D.

Table 2
Recovery of II B

Added (ug) ($n = 3$)	Recovered (ug)	% Recovery	%R.S.D.
	8.198	101.1	
8.112	8.390	103.4	1.5
	8.143	100.4	
	9.735	96.0	
10.14	9.988	98.5	1.6
	10.03	98.9	
	12.325	101.3	
12.168	12.032	98.9	1.2
	12.153	99.9	

n = Number of determinations.

3.2.3. Limit of detection (LOD) and limit of quantification (LOQ)

The detection sensitivity can be demonstrated by the LOD. A signal-to-noise ratio of approximately 2–3 is generally considered to be acceptable for estimating the detection limit, which is the lowest concentration that can be detected. The LOD of II B was found to be 2 μg/ml for six consecutive injections and %R.S.D. was found to be less than 15.

The quantification limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A signal to noise ratio between 9 and 11 is considered as LOQ. The LOQ of II B was found to be 7.5 μg/ml. For six consecutive injections the %R.S.D. was found to be less than 10.

3.2.4. Ruggedness

The ruggedness of a method is defined as degree of reproducibility of results obtained by analysis of

Table 3
II B accuracy

% Recovery Lab A ($n = 3$)	% Recovery Lab B
101.1	98.2
103.4	97.1
100.4	99.5
96.0	95.2
98.5	99.8
98.9	96.8
101.3	100.8
98.9	97.4
99.9	98.7

n = Number of determinations.

same sample under variety of normal test conditions such as different labs, different analysts, different instruments and different lots of reagents. The same samples analysed by preparing freshly in the laboratory B with a different instrument (Waters 510 HPLC delivery system, Waters 717 plus auto injector fitted with a waters 486 tunable absorbance detector). Linearity of the method was checked by preparing solutions freshly at concentration levels of 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0% for II B. The accuracy of the method was checked by spiking II B with II A at concentrations of 0.8, 1.0, and 1.2% of the analyte concentration (1.0 mg/ml) in triplicate. The data obtained from laboratory B agreed well with the results obtained in laboratory A (Waters Alliance 2690 separation module, fitted with a Waters 996 photodiode array detector). II B accuracy results are shown in Table 3.

3.2.5. Robustness

The robustness of a method is the ability to remain unaffected by small changes in mobile phase, temperature, and percentage organic modifier concentration. To determine robustness of the method experimental conditions were deliberately altered and chromatographic characteristics were evaluated. There was no significant change in resolution and peak shapes of II A and II B upon introduction of intentional variations in parameters such as basic strength of DEA (from 0.2 to 0.4%) and variation in organic modifier (IPA content) did not have significant effect on resolution of two enantiomers but retention time was altered. A slight decrease in retention time was observed with increase in the levels of isopropanol.

4. Conclusions

A normal-phase chiral LC method has been developed on a Chiralcel OD column for the separation of two enantiomers present in (±) *trans* (3RS, 4SR)-1-benzyl-4-(4-fluorophenyl)-3-hydroxymethylpiperidine (I). The method is simple, reproducible and sensitive. The separation was also obtained on Chiralcel OJ and Chiralpak AD columns. The method was validated using the Chiralcel OD column and has been found to be useful for routine process monitoring of II B content in II A.

Acknowledgements

The authors wish to thank the management of Dr Reddy's group for supporting this work and authors wish to acknowledge the Technology Development Centre of Dr Reddy's Laboratories Ltd for providing the samples for our research. We also express our thanks to the colleagues in separation science division of Dr Reddy's Discovery Research for their full co-operation in carrying out this work.

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