(Research Article)

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A Validated Chiral Liquid Chromatographic Method for The Enantiomeric Separation of Dapoxetine Hydrochloride

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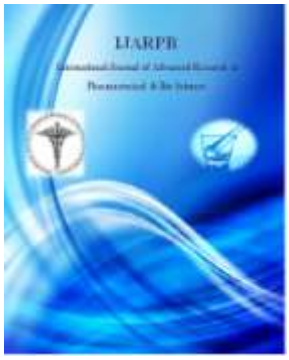

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ABSTRACT

A new and accurate chiral liquid chromatographic method was developed for the enantiomeric resolution of Dapoxetine hydrochloride, (*S*)-N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylPropan-1-amine, a premature ejaculation in bulk drugs. The enantiomer of Dapoxetine hydrochloride were baseline resolved on a Phenomenex Lux-cellulose-1 (250mm×4.6 mm, 5um) column using a mobile phase system containing hexane: 1-propanol: diethyl amine (97.5:2.5:0.1, v/v/v). The resolution between the enantiomer was not less than 3.5 and interestingly distomer was eluted prior to eutomer in the developed method. The presence of diethyl amine and 1-propanol in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was extensively validated and proved to be robust. The detection limit and quantitation limit of (*R*)-enantiomer were found to be 0.017% and 0.05%, respectively. The recovery of (*R*)-enantiomer was ranged from 90-110% in bulk drug samples. Dapoxetine hydrochloride sample solution and mobile phase were found to be stable for at least 48 h. The proposed method was found to be suitable and accurate for the quantitative determination of (*R*)-enantiomer in bulk drugs.

KEYWORDS: Dapoxetine hydrochloride, Cellulose-1, Chiral HPLC, Validation.

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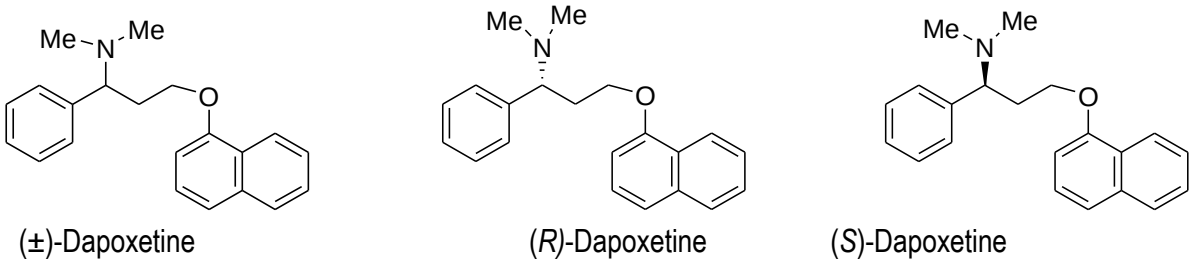
INTRODUCTION

Dapoxetine hydrochloride is a novel short-acting SSRI for the treatment of premature ejaculation (PE). In preclinical models, dapoxetine has been statistically shown to significantly inhibitory ejaculatory expulsion reflexes, acting at a supraspinal level [1-2]. Dapoxetine hydrochloride is designated chemically as (S)-N, N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine with empirical formula of $C_{21}H_{23}NO$ and molecular weight of 305.41[3]. Premature ejaculation is the most common form of male sexual dysfunction [4]. Premature ejaculation was considered a psychosomatic problem [5-6]. Dapoxetine hydrochloride is a short-acting SSRI, which may be better suited to the treatment of premature ejaculation [7]. The (S)-Dapoxetine is 3.5 times more potent than is (R)-Dapoxetine hydrochloride [8]. Very few methods are reviewed for Dapoxetine hydrochloride which reveals that high performance liquid chromatography method is described for the determination of Dapoxetine and its mono- and di-desmethyl metabolites in human plasma [9]. Development and validation of RP-HPLC method for the determination of Dapoxetine hydrochloride in pharmaceutical formulation using an experimental design [10]. As per our knowledge, there is no reference for the enantiomeric separation of Dapoxetine hydrochloride in bulk drugs using high performance liquid chromatography. The development of analytical methods for the quantitative analysis of chiral materials and for the assessment of enantiomeric purity is extremely challenging due to the fact that enantiomers posses virtually identical properties [11]. The report describes a chiral LC method for the enantiomeric separation of Dapoxetine hydrochloride using cellulose based chiral stationary phase, Lux cellulose-1 column. The developed HPLC method was validated for (*R*)-enantiomer in Dapoxetine hydrochloride.

MATERIALS AND METHODS

Dapoxetine hydrochloride and (*R)*-enantiomer were kindly supplied by Process Research Department of Troy Life Sciences Private Limited, Bangalore, India, HPLC grade hexane, 1-propanol and diethyl amine were purchased from Merck, Germany. The chemical structures are given in Fig. 1.



(±)-Dapoxetine (*R)*-Dapoxetine (*S*)-Dapoxetine

Fig. 1: Chemical structures of racemic and enantiomers of Dapoxetine

EQUIPMENT

A Shimadzu prominence HPLC system equipped with inbuilt auto injector, and photo diode array detector was utilized for method development and validation, Water´s Empower was used for data acquisition and system suitability calculations.

SAMPLE PREPARATION

The analyte concentration of Dapoxetine hydrochloride was fixed as 0.05 mg/ml. Working solutions of Dapoxetine hydrochloride and (R)-enantiomer were prepared in mobile phase. The Analytical method validation was performed with the specification limit of 0.5% level with respect to sample concentration.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions were optimized using a Lux cellulose-1 (250 X 4.6mm, 5u) Phenomenex make. The mobile phase was hexane: 1-propanol: diethyl amine (97.5:2.5:0.1, v/v/v). The flow rate was set at 0.8ml/min. The column was maintained at 25°C, and the detection was carried out at a wavelength of 230 nm. The injection volume was 20 µl. The run time was 25 min.

VALIDATION OF THE METHOD
METHOD REPRODUCIBILITY

Method reproducibility was determined by measuring repeatability and reproducibility (between system precision and Method precision) of retention times and peak area for each enantiomer in order to determine the repeatability of the method. Replicate injections (n=6) of a 0.05 mg/ml solution containing Dapoxetine hydrochloride spiked with (*R*)-enantiomer (0.5%) was carried out. The system precision and method precision was performed for six successive injections.

QUANTITATION LIMIT AND DETECTION LIMIT OF (R)-ENANTIOMER

The detection of limit, defined as lowest concentration of analyte that can be clearly detected above the baseline signal, was estimated as three times the signal to noise ratio. The Quantitation limit, defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, was estimated as ten times the signal to noise ratio. The detection limit (DL) and Quantitation limit (QL) were achieved by signal to noise ratio method.
The precision of the developed chiral method for (*R*)-enantiomer at Quantitation limit was checked by analyzing six test solutions of (*R*)-enantiomer prepared at QL level and calculating the percentage relative standard deviation of the area.

LINEARITY OF (R)-ENANTIOMER

Detector response linearity was assessed by preparing six calibration sample solution of (*R*)-enantiomer covering from QL level to 200% (50, 75, 100, 125, 150 and 200%), prepared in mobile phase from (*R*)-enantiomer stock solution. Regression curve was obtained by plotting peak area versus concentration. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

ACCURACY OF (R)-ENANTIOMER IN BULK SAMPLE

The Dapoxetine hydrochloride bulk sample, standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of (R)-enantiomer in bulk drug samples. The study was carried out in triplicate at 50, 75, 100, 125, and 150% of the Dapoxetine hydrochloride analyte concentration. The recovery of (*R*)-enantiomer was calculated.

ROBUSTNESS

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, mobile phase composition and column temperature. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between Dapoxetine hydrochloride and (*R*)-enantiomer was evaluated.

SOLUTION STABILITY AND MOBILE PHASE STABILITY

Stability of Dapoxetine hydrochloride in solution at analyte concentration was studied, and it is stable up to 48 hours.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The aim of this work is to separate the enantiomers of Dapoxetine hydrochloride and accurate quantification of (*R)*-enantiomer. 0.05 mg/ml solution of racemic mixture prepared in mobile phase was used in the method development. To develop a rugged and suitable LC method for the separation of Dapoxetine hydrochloride, different mobile phases were employed.

Various experiments were conducted to select the best mobile phase that would give optimum resolution and selectivity for the two enantiomers. The peak resolutions were found to be very poor when mobile phase consisting of hexane: isopropanol: diethyl amine (85:15:0.1, v/v/v) and the column were Lux cellulose-1 used. Introduction of 1-propanol in the mobile phase enhanced the chromatographic efficiency and resolution between the enantiomers. Very good separation was achieved on Lux cellulose-1 (resolution greater than 3.5). Due to the better chromatographic results obtained on the Lux cellulose-1 column, the method validation was carried out on the same. In the optimized method, the typical retention times of (*R*)-enantiomer and Dapoxetine hydrochloride were about 8.2 and 10.45 min, respectively. The enantiomeric separation of Dapoxetine hydrochloride by using isopropanol and 1-propanol in two separate mobile phases has been depicted in Fig. 2. The Resolution obtained for system suitability using isopropanol and 1-propanol was presented in Table 1.

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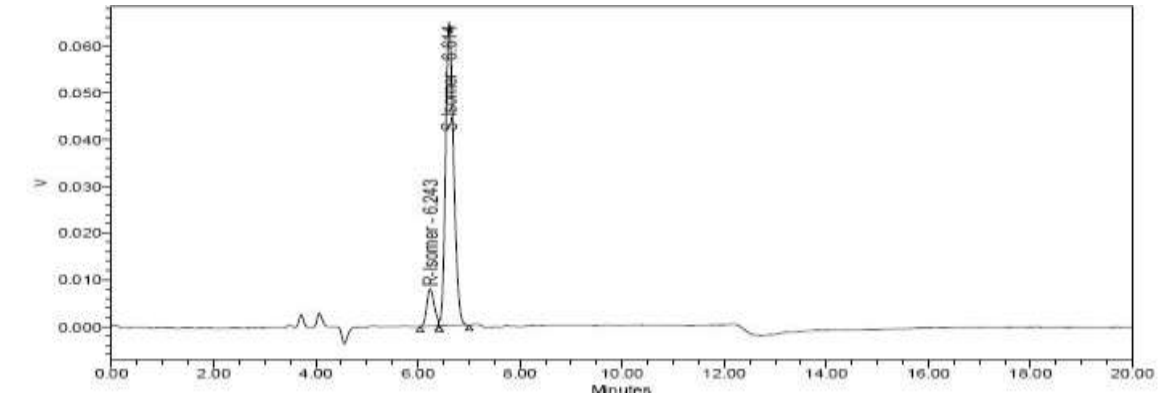
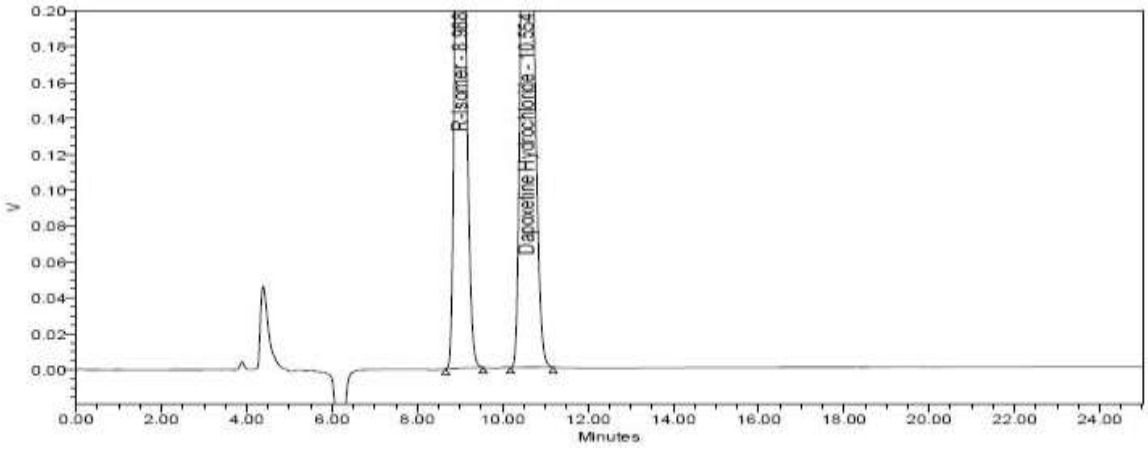


(A)



(B)

Fig. 2: Enantiomeric resolution of racemic Dapoxetine hydrochloride for (A) Lux cellulose-1 column and for (B) Lux cellulose-1 column. Mobile phase consisted for (A) hexane: isopropyl alcohol: diethyl amine (85:15:0.1 v/v/v), mobile phase consisted for (B) hexane: 1-propanol: diethyl amine (97.5:2.5:0.1, v/v/v); flow rate, 0.8ml/min; UV, 230 nm; column temperature, 25°C.

Table-1 System suitability report

Column name	Mobile phase	compound (n=3)	R_S	N	T
Luxcellulose-1	85:15:0.1	(R)-Enantiomer	1.26	3256	1.22
		(Dapoxetine)		3500	1.48
Luxcellulose-1	97.5:2.5:0.1	(R)-Enantiomer	3.56	7678	1.26
		(Dapoxetine)		7632	1.27

n=3 determinations; R_S, Resolution; N, number of theoretical plates; T, tailing factor

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VALIDATION RESULTS OF THE METHOD

In the repeatability study, the relative standard deviation (RSD) for retention time of Dapoxetine hydrochloride was 1.55 and for that of (R)-enantiomer was 2.62. Also, the relative standard deviation (RSD) for peak area of Dapoxetine hydrochloride was 1.14 and for that of (R)-enantiomer was 1.36 (Table 2). The limit of detection and limit of quantitation concentration were estimated as 0.00000875mg/ml and 0.000025mg/ml respectively for (R)-enantiomer, when signal-to-noise ratio of 3 and 10 were used as the criteria. The precision for (R)-enantiomer at quantitation limit was 0.2% R.S.D. Good linearity was observed for (R)-enantiomer over the concentration range of 0.000025 to 0.1mg/ml, with the linearity correlation coefficient, R^2=0.999 (Table 2) and Linearity curve shown in (Fig. 3.). The standard addition and recovery experiments were conducted for (R)-enantiomer in bulk samples in triplicates at 50%, 75%, 100%, 125%, and 150% of analyte concentration. Percentage of recovery was calculated and the results were ranged from 99 to 101 (Table 3).

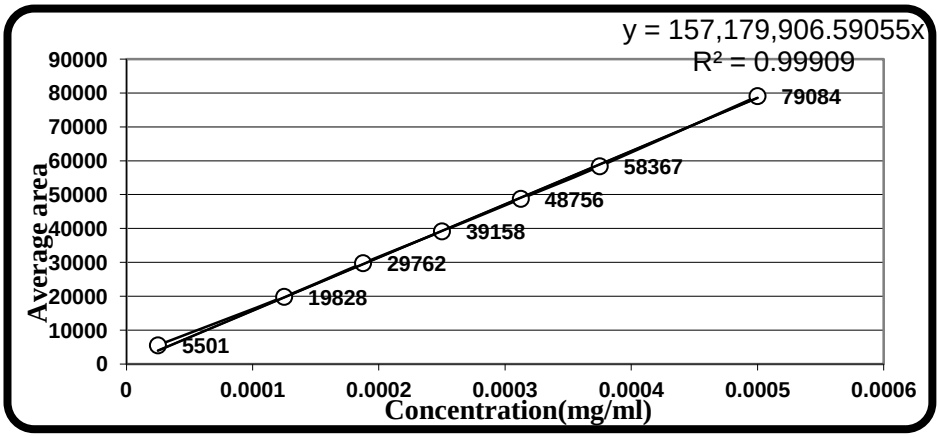

Fig. 3. Linearity curve of (R)-enantiomer of Dapoxetine hydrochloride.

Table-2: Validation results of the developed chiral LC method

Validation Parameter	Results
Repeatability (n=6, % RSD)	
Retention time (S-enantiomer)	0.20
Area (S-enantiomer)	0.27
Reproducibility (n=6, %RSD)	
Retention time (R-enantiomer)	2.62
Retention time (S-enantiomer)	1.55
Area (R-enantiomer)	1.36
Area (S-enantiomer)	1.14
DL-QL (R-enantiomer)	
Detection limit (%)	0.017

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Quantitation limit (%)	0.050
Precision at QL (% RSD)	0.88
Accuracy at QL (n=3)	
% Recovery	100.14
Linearity (R-enantiomer)	
Calibration points	7
Correlation coefficient	0.999

Table-3 Recovery results of (R)-enantiomer in bulk drugs

Validation Parameter	% Recovery	% RSD
Accuracy (n=3)		
50% solution	100.00	0.19
75% solution	100.60	0.97
100% solution	99.37	0.39
125% solution	99.67	0.07
150% solution	100.38	0.29

n=3 determinations.

HPLC chromatogram of spiked (*R*)-enantiomer at 0.5% level in Dapoxetine hydrochloride bulk drug sample was shown in Fig.4 the chromatographic resolution of Dapoxetine hydrochloride and (*R*)-enantiomer peaks was used to evaluate the method robustness under modified conditions. The resolution between Dapoxetine hydrochloride and (R)-enantiomer was greater than 4.0, under all separation conditions tested (Table-4), demonstrating sufficient robustness.

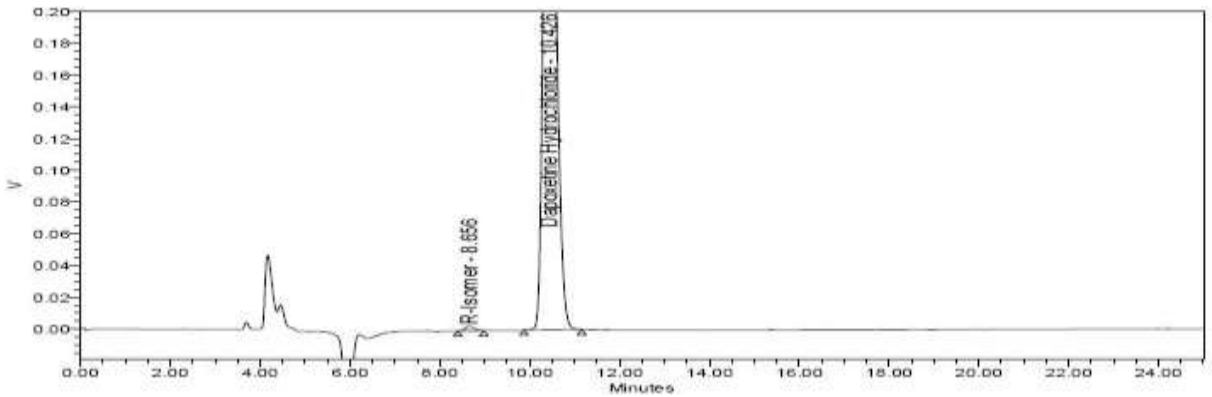

Fig. 4. Typical HPLC chromatogram of Dapoxetine hydrochloride bulk sample (0.05 mg/ml) Spiked with (*R*)-Enantiomer (0.5%).

Table-4 Robustness of the chiral LC method

Validation Parameter	Resolution between Dapoxetine hydrochloride and (R)-enantiomer
Flow rate (ml/min)	
0.8	4.22
1.0	4.13
1.2	3.96
Column temperature (°C)	
25	4.19
30	3.98

No significant change in the (*R*)-enantiomer content was observed in Dapoxetine hydrochloride sample during solution stability and mobile phase stability experiments. Hence, Dapoxetine hydrochloride sample solution and mobile phase solution are stable for at least 48 h.

CONCLUSION

A new and accurate normal phase chiral LC method was described for the enantiomeric separation of Dapoxetine hydrochloride. Cellulose-based chiral column Lux cellulose-1 column was found to be selective for the enantiomer of Dapoxetine hydrochloride. Method validation was carried out using the Lux cellulose-1 column due to the better chromatographic results achieved in this column. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be used for the quantitative determination of chiral impurity (R)-enantiomer in bulk materials.

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