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A VALIDATED NORMAL PHASE CHIRAL LC METHOD FOR THE ENANTIOMERIC SEPARATION OF SERTRALINE AND ITS Cis- (1*R*, 4*R*) ENANTIOMER ON AMYLOSE BASED STATIONARY PHASE

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ABSTRACT

A simple and rapid chiral liquid chromatographic method was developed for the enantiomeric separation of Sertraline hydrochloride (cis-(1*S*, 4*S*)-4-(3, 4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride) and its undesired cis-enantiomer (cis- (1*R*, 4*R*)-4-(3, 4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride). Superior resolution between Sertraline and its cis-(1*R*, 4*R*) enantiomer was achieved on amylase based Chiralpak AD-H (250 x 4.6 mm, 5 µm particle size) column using hexane, isopropyl alcohol, ethanol and diethyl amine (850:100:50:0.1 v/v/v/v) as mobile phase at 25 °C temperature. Flow rate was kept as 1.0 ml/min and elution was monitored at 215 nm. The sample concentration was 0.3 mg/ml. The effects of the mobile phase composition, the flow rate and the temperature on the chromatographic separation were investigated. Developed method is capable to detect (LOD) and quantitate (LOQ) cis-(1*R*, 4*R*) enantiomer to the levels of 30 and 120 ng/ml respectively, for 10 µl injection volume. The percentage RSD of the peak area of six replicate injections of cis-(1*R*, 4*R*) enantiomer at LOQ concentration was 4.9. The percentage recoveries of cis-(1*R*, 4*R*) enantiomer from Sertraline were ranged from 93.8 to 103.9. The test solution and mobile phase was observed to be stable up to 24 h after the preparation. The developed method was validated with respect to limit of detection (LOD), limit of quantitation (LOQ), precision, linearity, accuracy, robustness and ruggedness.

Key words :High performance liquid chromatography,Enantiomers,Validation and quantification,Sertraline

INTRODUCTION

Understanding the stereochemistry of pharmaceutical compounds is very important in regard to their biological activities within human body. In 1992, Food and Drug Administration (FDA) has issued a policy statement for the development of new stereoisomeric drugs that requires the acceptable manufacturing control of synthesis and impurities, adequate pharmacological and toxicological assessment, proper characterization of metabolism and distribution, and appropriate clinical evaluation [1].

Sertraline hydrochloride (cis-(1*S*, 4*S*)-4-(3, 4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride) (Fig-1) is an antidepressant for oral administration. It is chemically unrelated to tricyclic, tetracyclic, or other available antidepressant agents. Sertraline hydrochloride is a novel drug substance belonging to the group of selective serotonin (5-hydroxitriptamine, 5-HT) reuptake inhibitors (SSRIs) in the brain [2]. Clinical studies in man have shown that sertraline blocks the uptake of serotonine into human platelets. Sertraline is a very selective inhibitor of serotonine reuptake, having only weak effects on other monamines such as norepinephrine and dopamine and is four to five times more potent than fluoxetine in blocking serotonine reuptake. Since, there is a chance of formation of potential cis-(1*R*,

4*R*) enantiomer (cis- (1*R*, 4*R*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride) (Fig-1) during the synthesis of Sertraline. Chromatographic separation of cis-(1*R*, 4*R*) enantiomer from Sertraline and followed by its quantitative determination is one of the key quality parameter before Sertraline is released to market.

A electrokinetic chromatography using cyclodextrin as chiral selector in the mobile phase method for the separation of cis-trans isomers and enantiomers of Setraline was reported in the literature [3, 4]. A stability-indicating chiral liquid chromatography for determination of related substances and chiral purity of Sertraline was reported in the literature [5]. Analysis of cis-trans isomers and enantiomers of Setraline by using the beta cyclodextrin-modified miceller electrokinetic chromatography was reported in the literature [6]. HPLC determination of sertraline in bulk drug, tablets and capsules using hydroxypropyl-β-cyclodextrin as mobile phase additive was reported [7, 8]. Quantitative 1H NMR method for the routine spectroscopic determination of enantiomeric purity of active pharmaceutical ingredients fenfluramine, sertraline, and paroxetine [9]. Reported methods are mostly developed based on beta cyclodextrin as mobile phase additive and using the electrokinetic chromatography. One of the liquid chromatographic method was reported the higher quantitation levels using the typical pH adjusted high concentrated phosphate buffer. So far to our knowledge no chiral normal phase liquid chromatographic method for determination of potential cis-(1*R*, 4*R*) enantiomer of Sertraline in Sertraline bulk drug samples has been reported.

The present research work focused on to develop the simple and rapid normal phase chiral HPLC method for the determination cis-(1*R*, 4*R*) enantiomer content in bulk drug samples of Sertraline hydrochloride using commercially available chiral stationary phases. Superior resolution between cis-(1*R*, 4*R*) enantiomer and Sertraline was observed on amylase based Chiralpak AD-H column (USP resolution > 7.5) within the short run time using simple normal phase system containing a mixture of hexane, isopropyl alcohol, ethanol and diethyl amine. This paper also deals method validation of developed method.

EXPERIMENTAL

Chemicals and Reagents:

Samples of Sertraline hydrochloride and cis-(1*R*, 4*R*) enantiomer (Fig. 1) samples were obtained from the Process development laboratory of Bulk Actives Unit-III, a business unit of Dr. Reddy's Laboratories Ltd., Hyderabad, India.

Analytical reagent grade potassium dihydrogen phosphate, HPLC grade hexane and diethyl amine (DEA) was purchased from Qualigens fine chemicals, Mumbai, India, acetonitrile, ethanol and isopropyl alcohol was purchased from Ranbaxy fine chemicals, New Delhi, India and HPLC grade water was produced internally by using Milli-Q, Millipore water purification system

Instrumentation:

Waters make HPLC (Alliance 2690 Model, Waters Corporation, Milford, USA) equipped with 2695 separation module with inbuilt auto injector and 2487 dual wavelength absorbance detector was used for the analysis, The output signal was monitored and processed using Empower software (Waters) on Pentium computer (Digital Equipment Co) (Laboratory A).

Agilent make 1200 series HPLC (Agilent Technologies Inc., Palo Alto, CA, USA) system equipped with diode array detector was used for ruggedness and peak homogeneity verification. The output signal was monitored and processed using Chemstation software on Pentium computer (Digital Equipment Co) (Laboratory B).

Sample preparation:

15 mg of each Sertraline hydrochloride (99.6% pure) and its cis- (1*R*, 4*R*) enantiomer (99.3% pure) of Sertraline were dissolved in 50 mL of ethanol (diluent) to get individual concentrations of 0.3 mg/ml and 0.1 mg/ml respectively in the mixture. This solution was used for the enantiomeric separation. The target analyte concentration of Sertraline was fixed as 0.3 mg/ml.

RESULTS AND DISCUSSION

Method development:

The aim of this work was to develop the simple and rapid method to determine the content of cis-(1*R*, 4*R*) enantiomer in Sertraline hydrochloride bulk drug samples. The mixture of Sertraline and its cis-(1*R*, 4*R*)

enantiomer solution was used in the method development. During the method development different chiral stationary phases (CSPs) were selected that are commercially available in the market. Different chiral stationary phases are namely Chiralpak IC, Chiralpak IA, Chiralpak AS-H, (R, R) Whelk-01, Chiralcel OJ-H, Chiralcel OD-RH and Chiralpak AD-H. Different trials were made during the method development and the details were listed in the Table 1.

Optimized Chromatographic Conditions:

Good chromatographic separations were achieved only on amylase based Chiralpak AD-H (250 x 4.6 mm, 5 µm particle size, Make: Daicel Chemical Industries Ltd, Japan) chiral column using the mobile phase system contains the mixture of hexane, isopropyl alcohol, ethanol and diethyl amine (850:100:50:0.1 v/v/v/v). The flow rate was kept at 1.0 ml/min. The sample concentration was 0.3 mg/ml in diluent (ethanol). The column temperature was maintained at 25 °C and the elution was monitored at 215 nm. The injection volume was 10 µl.

Good separations were observed within short runtime on amylase based Chiralpak AD-H column (*USP* resolution > 7.5). The typical retention times of Sertraline and cis-(1*R*, 4*R*) enantiomer were 4.8 and 6.8 min, respectively (Fig. 2). The system suitability [10] results were listed in Table 2.

Method Validation:

Limit of Detection and Limit of Quantitation

The limit of detection (LOD), defined as smallest amount of analyte that can be clearly detected above the baseline, was estimated as the amount for which the signal to noise ratio was 3 [11]. The limit of detection for cis-(1*R*, 4*R*) enantiomer was found to be 30 ng/ml for 10 µL of injection volume. The limit of quantitation (LOQ), defined as lowest concentration of analyte that can be quantified with acceptable precision and accuracy, was estimated as the amount for which signal to noise ratio was 10 [11]. The limit of quantitation for cis-(1*R*, 4*R*) enantiomer was found to be 120 ng/ml for 10 µL of injection volume. The precision for cis-(1*R*, 4*R*) enantiomer at LOQ level was good, the relative standard deviation was found to be 4.9 %. Results were listed in Table 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions [12]. The precision was evaluated by calculating the percentage of RSD of six determinations by injecting six freshly prepared solutions containing cis-(1*R*, 4*R*) enantiomer of Sertraline at three concentration levels viz. 0.05, 0.10 and 0.15% with respect to analyte concentration (0.3 mg/ml) and %RSD values were found to be 4.7, 4.2 and 3.5 respectively. For intra-day precision, the sample at the above three concentration levels was injected on three different days and the %RSD values were found to be 5.1, 4.7 and 3.5. The low RSD% values are confirms the good precision of the developed method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to furnish responses, which are directly proportional to the concentration of the analyte in the sample [13]. . The linearity of the method was checked at six concentration levels i.e. from LOQ to 1000 ng/ml of cis-(1*R*, 4*R*) enantiomer of Sertraline. The coefficient of regression of the calibration curve was found to be 0.998, reveals that an excellent correlation existed between the peak area and concentration of the cis-enantiomer.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the method for quantification of the cis-(1*R*, 4*R*) enantiomer in bulk drug samples. The accuracy of the method was determined by spiking cis-(1*R*, 4*R*) enantiomer to Sertraline at 120 (LOQ), 300 and 600 ng/ml levels with the three batches of Sertraline. The percentage recoveries were ranged from 93.8 to 103.9 in samples of Sertraline.

Ruggedness and Robustness

The ruggedness [14] of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under variety of normal test conditions such as different laboratories, different analysts, different instruments, different days and different lots of reagents. The standard addition and recovery experiments of cis-(1*R*, 4*R*) enantiomer was carried out in Sertraline bulk samples at the same

concentration levels tested in Laboratory A were again carried out at laboratory B using a different instrument by a different analyst. The data obtained from Laboratory B was well in agreement with the results obtained in Laboratory A, thus proving the method ruggedness.

The robustness [11] of an analytical procedure is measure of its capability to remain unaffected by small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions like flow rate, mobile phase ratio and column temperature, the resolution between the peaks of cis-(1*R*, 4*R*) enantiomer and Sertraline was found to be > 7.4 illustrating the robustness of the method. Robustness results were listed in Table 4.

Solution stability and mobile phase stability

Solution stability was studied by keeping the test solution in tightly capped volumetric flask at room temperature on a laboratory bench for 24 h. Content of cis-(1*R*, 4*R*) enantiomer was checked for every 6 h interval and compared with freshly prepared solution. No variation was observed in the content of cis-(1*R*, 4*R*) enantiomer for the study period and it indicates Sertraline sample solutions prepared in diluent were stable up to 24 h.

Mobile phase stability was carried out by evaluating the content of cis-(1*R*, 4*R*) enantiomer in Sertraline test sample solutions, which were prepared freshly at every 6 h interval for 24 h. The same mobile phase was used during the study period. No variation was observed in the content of cis-(1*R*, 4*R*) enantiomer for the study period and it indicates prepared mobile phase was found to be stable up to 24 h.

CONCLUSION

A simple and rapid isocratic normal phase chiral HPLC method was developed for enantiomeric separation and accurate quantification of cis-(1*R*, 4*R*) enantiomer from Sertraline. The limit of quantification is 120 ng/ml. The developed method was found to be selective for cis-(1*R*, 4*R*) enantiomer and Sertraline. The concentration of ethanol and DEA in the mobile phase plays an important role on enhancing the chromatographic efficiency and resolution between the enantiomers. Developed 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 cis-(1*R*, 4*R*) enantiomer content in Sertraline bulk drug samples.

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Table-1: Results of various trials

Trial No.	HPLC conditions	Remarks
1.	Column: chiralcel OD-RH 150 x 4.6 mm, 5 µm Mobile phase: 10 mM KH2PO4 and Acetonitrile (60:20, v/v) Flow rate: 1.0 ml/min Column temperature: 25 °C	Poor resolution between pair of enantiomer peaks (Rs <1.5)
2.	Column: chiralpak IC 250 x 4.6 mm, 5 µm Mobile phase: hexane and IPA (80:20, v/v) Flow rate: 1.0 ml/min Column temperature: 25 °C	Sertraline and cis-(1*R*, 4*R*) enantiomer peaks are co-eluted and observed broad peak shape
3.	Column: (R, R) Whelk-01 250 x 4.6 mm, 5 µm Mobile phase: hexane and IPA (80:20, v/v) Flow rate: 1.0 ml/min Column temperature: 25 °C	Sertraline and cis-(1*R*, 4*R*) Enantiomer peaks are co-eluted and observed broad peak shape
4.	Column: chiralpak AS-H 150 x 4.6 mm, 5 µm Mobile phase: hexane and IPA (80:20, v/v) Flow rate: 0.7 ml/min	Sertraline and cis-(1*R*, 4*R*) peaks are co-eluted and observed and observed broad peak shape

	Column temperature: 25 °C	
5.	Column: chiralcel OJ-H 250 x 4.6 mm, 5 µm Mobile phase: hexane and IPA (90:10, v/v) Flow rate: 0.7 ml/min Column temperature: 25 °C	No resolution and observed broad peak shape
6.	Column: chiralpak IA 250 x 4.6 mm, 5 µm Mobile phase: hexane and ethanol (90:10, v/v) Flow rate: 1.0 mL min $^{-1}$ Column temperature: 25 °C	No resolution and observed broad peak shape
7.	Column: chiralpak AD-H 250 x 4.6 mm, 5 µm Mobile phase: hexane and IPA (75:25, v/v) Flow rate: 0.8 ml/min Column temperature: 25 °C	Poor resolution between pair of enantiomer peaks (Rs <1.5) with *USP* tailing >2.5
8.	Column: chiralpak AD-H 250 x 4.6 mm, 5 µm Mobile phase: hexane, IPA and ethanol (80:10:10, v/v) Flow rate: 1.0 ml/min Column temperature: 25 °C	Good resolution between pair of enantiomer peaks (Rs <3.0) with *USP* tailing >1.9
9.	Column: chiralpak AD-H 250 x 4.6 mm, 5 µm Mobile phase: hexane, IPA, ethanol and DEA (85:10:5:0.1 v/v) Flow rate: 1.0 ml/min Column temperature: 25 °C	Superior resolution between pair of enantiomer peaks (Rs >7.5) *USP* tailing <1.5

Table-2: System suitability results

Name method	**Retention time (t_R) in min**	**Resolution (R_s) by Tangent**	***USP* Tailing factor (*T*)**	**No. of theoretical plates (*N*) USP tangent**
Sertraline	4.8	-	1.4	8461
cis-(1*R*, 4*R*) enantiomer	6.8	8.2	1.4	12284

Table-3:Precision results of cis-(1*R*, 4*R*) enantiomer at LOQ level

Preparation	**Peak area**
1.	7896
2.	7561
3.	7657
4.	7992
5.	7027
6.	7348
%RSD	4.9

Table-4: Robustness of the method

Parameter	**USP resolution between Sertraline and cis-(1*R*, 4*R*) enantiomer**
Flow rate (ml/min)	
0.8	8.7
1.0	8.2
1.2	7.6
Column temperature (°C)	

20	8.5
25	8.2
30	7.8
Ethanol percentage in mobile phase	
4	8.9
5	8.2
6	7.5

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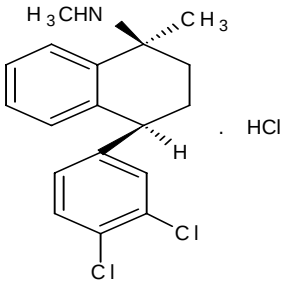
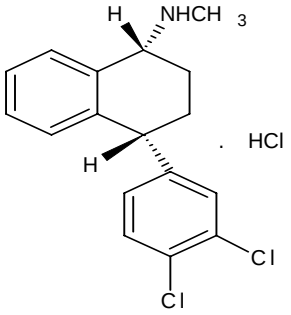


(a) Sertraline hydrochloride: cis-(1*S*, 4*S*)-4-(3, 4-dichlorophenyl)-*N*-dimethyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride



(b) cis-(1*R*, 4*R*) enantiomer: cis-(1*R*, 4*R*)-4-(3, 4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen -1-amine hydrochloride

Fig.-1: Chemical structures of Sertraline hydrochloride and cis-(1*R*, 4*R*) enantiomer

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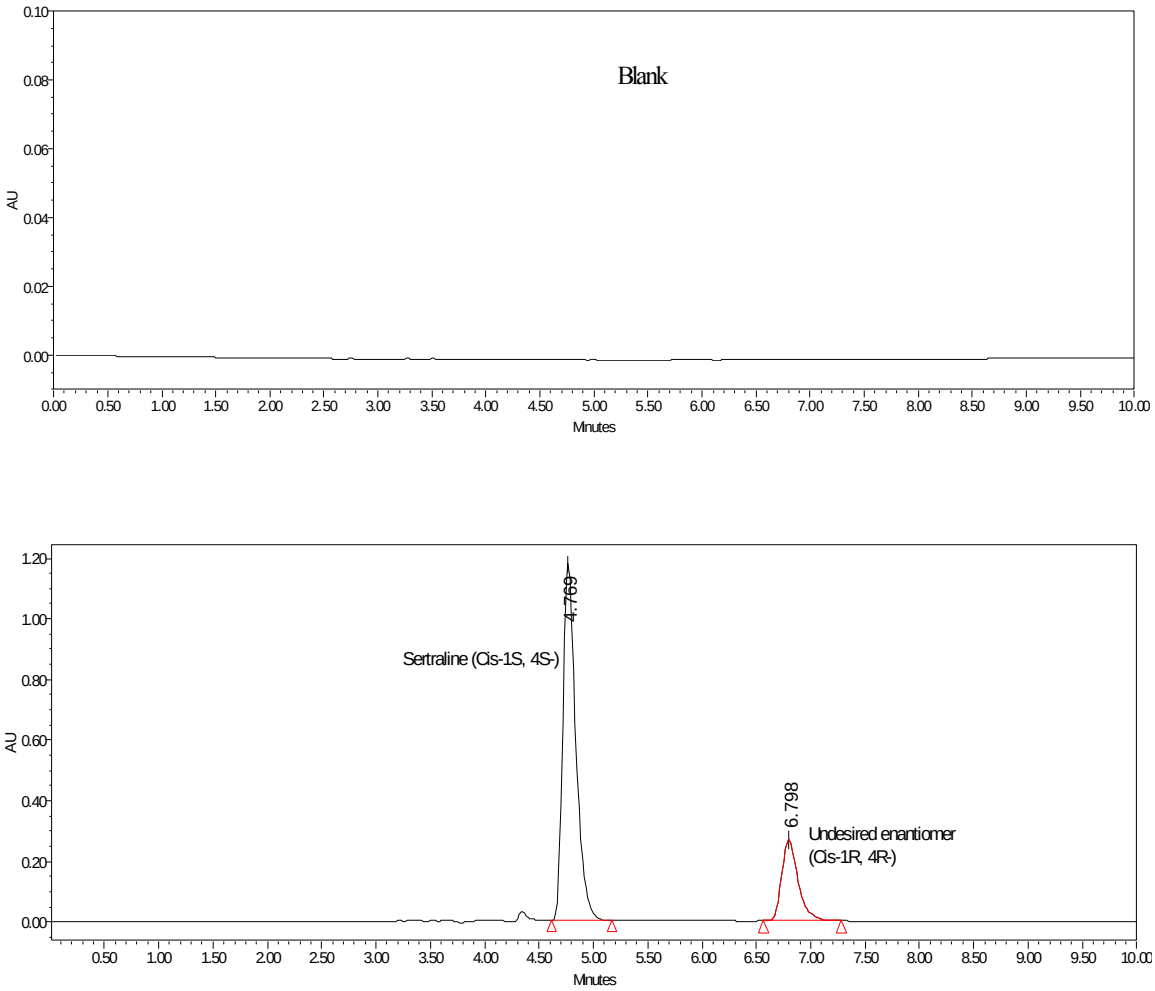

Fig.-2: Typical blank, Sertraline and cis-(1*R*, 4*R*) enantiomer spiked HPLC chromatograms

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"Imagination is the beginning of creation. You imagine what you desire, you will what you imagine and at last you create what you will."

- George Bernard Shaw