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A validated chiral LC method for the enantiomeric separation of sertraline hydrochloride in bulk drug samples and pharmaceutical dosage forms

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ABSTRACT

A simple and new isocratic polar mode chiral HPLC method has been developed for the enantiomeric separation of sertraline hydrochloride in bulk drugs and dosage forms with an elution time of about 15 min. The separation was achieved on immobilized amylose based chiral stationary phase (Chiralpak-IA) using 0.1% diethylamine in methanol as mobile phase. The mobile phase was delivered at 0.7 mL min^{-1} flow and the detection was monitored at 220 nm using ultraviolet detection technique. The resolution (R_S) between the sertraline and its (R, R)-enantiomer was found to be more than 4.0. The method shows 0.005µg as limit of detection (LOD) and 0.015µg as limit of quantification (LOQ) for (R, R)-sertraline, for 10µL injection volume. The validated method yield good results regarding precision, linearity and accuracy. The developed method shows excellent linearity (R^2 >0.999) over a range of LOQ to 0.3% for (R, R)-sertraline. The percentage recovery of (R, R)-sertraline ranged from 98.3 to 101.8 in bulk drug samples and in pharmaceutical dosage forms. Robustness studies were also carried out on the develop method. The sertraline hydrochloride sample solution stability and mobile phase stability studies were carried out and the results were found to be satisfactory for a study period of 48h.

KEYWORDS

sertraline hydrochloride;
Chiral HPLC;
Enantiomeric separation;
Validation;
Quantification.

INTRODUCTION

sertraline hydrochloride, the active ingredient in Serlift® is an anti depressant for oral administration. sertraline hydrochloride described chemically as (*cis*-(1*S*, 4*S*)-N-methyl-4-(3, 4-dichlorophenyl)-1, 2, 3, 4-tetrahydro-1-naphthalenamine hydrochloride) (Figure 1) is chiral in nature. It is chemically unrelated to tricyclic, tetracyclic, or other available antidepressant agents[1]. sertraline hydrochloride is a novel drug substance belonging to the group of selective serotonin reuptake inhibitors (SSRIs) in the brain[2]. It is used to treat depression, panic disorder, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), social anxiety disorder (social phobia), and a severe form of premenstrual syndrome called premenstrual dysphoric disorder (PMDD)[3-5]. sertraline hydrochloride works by helping to restore the balance of certain natural substances in the brain known as neurotransmitters.

Enantiomers of racemic drugs often differ in pharmacokinetic behaviour or pharmacological action[6]. The

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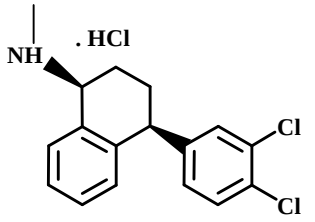
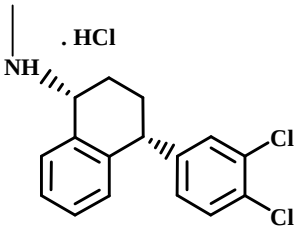


Sertraline Hydrochloride

cis-(1S, 4S)-N-methyl-4-(3, 4-dichlorophenyl)-1, 2, 3, 4-tetrahydro-1-naphthalenamine hydrochloride



(R, R) Sertraline Hydrochloride

cis-(1R, 4R)-N-methyl-4-(3, 4-dichlorophenyl)-1, 2, 3, 4-tetrahydro-1-naphthalenamine hydrochloride

Figure 1: Structures and labels of sertraline hydrochloride, (R, R) sertraline hydrochloride

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[7]. Although many analytical techniques can be employed to achieve this, the most widely used is liquid chromatography (LC) employing a chiral stationary phase (CSP)[8-10].

A few analytical methods has been reported in the literature for analysis of sertraline include determination of sertraline in bulk drug, tablets and capsules using hydroxypropyl-β-cyclodextrin as mobile phase additive[11], quantification of sertraline stereoisomers by electrokinetic chromatography[12], analysis of *cis-trans* isomers and enantiomers of sertraline by cyclodextrin-modified micellar electrokinetic chromatography[13] and rapid and sensitive method for the determination of sertraline in human plasma using LC-MS[14]. To the best of our knowledge till date there are no chiral HPLC methods available for enantiomeric separation of sertraline hydrochloride in bulk drugs and dosage forms by using immobilized chiral stationary phase.

Due to the chiral nature of the drug it is felt necessary to develop a simple and enantioselective HPLC method for the enantiomeric separation and accurate quantification of undesired enantiomer ((R, R)-sertraline) of sertraline hydrochloride. This paper deals with method development and validation of enantiomeric separation of sertraline hydrochloride.

EXPERIMENTAL

Chemicals

Samples of sertraline hydrochloride and its enantiomer were obtained from Reference Standard Laboratory of United States Pharmacopeia-India (P) Limited, Hyderabad, India. Commercially available 25 mg sertraline hydrochloride tablets (Serlift®) were purchased from Ranbaxy laboratories limited, India. HPLC grade n-hexane, isopropyl alcohol, diethylamine, methanol, acetonitrile and ethanol were purchased from Merck, Darmstadt, Germany.

Equipment

The HPLC system employed in the method development and validation was Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) LC system with a diode array detector (DAD). The output signal was monitored using Chemstation software (Agilent) on Pentium computer (Digital Equipment Co., Hoston, USA.)

The chiral columns used in method development were Chiralcel OD-H (cellulose tris (3,5-dimethylphenyl carbamate) coated onto silica-gel), Chiralpak AD-H (amylose tris (3,5-dimethylphenylcarbamate) coated onto silica-gel) and Chiralpak-IA (amylose tris (3,5-dimethylphenylcarbamate) immobilized onto silica-gel)[15]. All are of Daicel make (Daicel Chemical Industries Ltd., Japan) with 5μm particle size in (250×4.6) mm dimension.

Chromatographic conditions

The chromatographic conditions were optimized using a Chiralpak IA column. The mobile phase contains a 0.1% diethylamine in methanol. The flow rate of the mobile phase was 0.7 mL min^{-1}. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 220 nm. The injection volume was 10 μl. Mobile phase was used as diluent.

Preparation of standard solutions

Stock solutions of sertraline hydrochloride and (R, R)-sertraline (1000μg mL^{-1}) were prepared individually by dissolving the appropriate amount of the substances in the mobile phase. Working solutions of sertraline hydrochloride and (R, R)-sertraline were pre-

pared in diluent.

Preparation of sample solution

Twenty tablets were individually weighed to get the average weight of the tablets and powdered in mortar. A sample of the powdered tablets, equivalent to 10 mg of active pharmaceutical ingredient (sertraline hydrochloride) was transferred into 100 mL volumetric flask. About 75 mL of mobile phase was added and kept on rotatory shaker for 10 min to dissolve the material completely and sonicated for 10 min and diluted to 100mL. The content was centrifuged for 10 min at 3,000 rpm. The supernatant was collected and filtered using 0.45μnylon 66-membrane filter. The filtrate was used as stock solution.

Method validation

As per the ICH guidelines the method was validated in terms of following parameters[16, 17].

System suitability test

System suitability test is an integral part of chromatographic methods and is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed[18].

Precision

The precision of an analytical procedure express the closeness of agreement between a series of measurements from multiple sampling of the same homogeneous sample under prescribed conditions. The precision of the developed method was checked by injecting six individual preparations of (100μg mL^{-1}) sertraline hydrochloride spiked with 0.15% of its (R, R)-enantiomer. The percentage relative standard deviation of area for (R, R)-sertraline was calculated.

The intermediate precision of the method was also evaluated using different lot of column, on a different instrument, by different analyst on the same instrument, in different laboratories and the percentage relative standard deviation for six individual spiked preparations was calculated.

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

The LOD and LOQ for (R, R)-sertraline was estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study was carried at the LOQ level by injecting six individual preparations of (R, R)-sertraline and calculated the percentage relative standard deviation of area.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte sample. Linearity test solutions were prepared by diluting the (R, R)-sertraline (1000μg mL^{-1}) to the required concentrations. The solutions were prepared at seven concentration levels from LOQ to 200% of the permitted maximum level (0.15%). (LOQ, 0.0375, 0.075, 0.1125, 0.15, 0.225 and 0.3%). The peak area versus concentration of (R, R)-sertraline was subjected to regression analysis to calculate calibration equation and correlation coefficient.

Accuracy

Standard addition and recovery experiments were conducted to determine accuracy of the method for the quantification of (R, R)-sertraline of sertraline hydrochloride in bulk drug sample and in pharmaceutical dosage forms.

The study was carried out in triplicate at 0.075%, 0.15% and 0.225% of the target analyte concentration (100μg mL^{-1}). The percentage recovery of (R, R)-sertraline was calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between sertraline and (R, R)-sertraline was checked. The flow rate of the mobile phase was 0.7 mL min^{-1}. To study the effect of flow rate on the resolution of enantiomers, it was changed by 0.1 units from 0.7 mL min^{-1} (i.e 0.6 mL min^{-1} to 0.8 mL min^{-1}). The effect of column temperature on resolution was studied at 23 and 27^{0}C instead of 25°C. In the all above varied conditions, the components of the mobile phase were held constant as that of initial. The effect of change in %diethyl amine on the resolution was studied by changing from -0.05 to +0.05%. The resolution between enantiomers was evaluated in all the above experimental conditions.

Solution stability and mobile phase stability

The solution stability of sertraline hydrochloride and its(R, R)-enantiomer was carried out by leaving spiked

sample solution in tightly capped volumetric flask at room temperature for 48h. Content of(R,R)-sertraline was determined for every 6h interval and compared with freshly prepared solution at each time point.

Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6h interval. Content of (R, R)-sertraline was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

Method development and optimization

The aim of this work is to separate the enantiomers of sertraline hydrochloride and accurate quantification of (R, R)-enantiomer of sertraline. The mixture of sertraline hydrochloride and its (R, R)-enantiomer were used during the method development. To develop a rugged and suitable LC method for the enantiomeric separation of sertraline hydrochloride different mobile phases and stationary phases were employed. In the method development various chiral columns namely Chiralcel-ODH, Chiralpak-ADH and Chiralpak-IA were employed. Various experiments were conducted to select the best stationary and mobile phases that would give optimum resolution and selectivity for the enantiomers. The preliminary trials were carried on poly saccharide type chiral columns namely Chiralcel OD-H and Chiralpak AD-H of Daicel make. Poor separation was observed on Chiralcel OD-H and Chiralpak AD-H while using the n-hexane: ethanol (75:25, v/v) as mobile phase. Marginal separation was found on the Chiralpak-ADH while using n-hexane: isopropyl alcohol (75:25, v/v) as mobile phase. Due to the presence of basic moiety in sertraline hydrochloride 0.1% of diethyl amine (DEA) is introduced to the above mobile phase and found not much improvement in selectivity. Due to limited solvent compatibility in coated type polysaccharide columns we gave up the further optimization on Chiralpak AD-H column. Then we tried with immobilized type chiral stationary phase (CSP) Chiralpak-IA by using polar solvent as mobile phase. Different experiments have been conducted by using different solvents like 100% acetonitrile, ethanol and methanol. The separation is not satisfactory with 100% acetonitrile. There is an indication of better separation was observed with 100% ethanol (R_s~2.7) but peak tailing was more (USP tailing ~1.9). Introduction of 0.1% DEA to the above mobile phase increased the resolution between the enantiomers (R_s~3.1) and controlled the peak tailing (USP tailing ~1.5) but the S/N ratio was not satisfactory and back pressure also high. Better separation was achieved with 100% methanol (R_s~3.1) and peak tailing was marginally low (USP tailing ~1.3). Introduction of 0.1% DEA to the above mobile phase increased the resolution (R_s~4.0) and reduced the peak tailing (USP tailing ~1.0). The S/N ratio was satisfactory and back pressure was comparatively low. Finally the best separation was noticed with the mobile phase consisting of 0.1% DEA in methanol. The typical retention times of sertraline hydrochloride and its (R, R)-enantiomer were about 6.0 and 7.0 min respectively (Figure 2). The system suitability results are given in TABLE 1. Due to the better chromatographic results obtained on the Chiralpak-IA column and due to better column life, the method validation was carried out on this column.

TABLE 1: System suitability test results

Name	Retention time(t_r)in min	Resolution (R_S)	No. of theoretical plates (N)	Tailing factor(T)
Sertraline hydrochloride	6.0	-	15998	1.0
(*R, R*) Sertraline	7.0	4.1	9621	1.0

The chiral stationary phase (CSP) present in Chiralpak-IA column is amylose tris (3,5-dimethyl phenylcarbamate) immobilized onto silica gel. The separation of enantiomers on Chiralpak-IA column could be due to the interaction between the solute enantiomers and polar carbamate group (-HN-C=O) on the CSP. The carbamate group on CSP can interact with solute enantiomers through hydrogen bonding using C=O and NH group which are present in CSP and sertraline hydrochloride.

Method validation

1. Precision

The %RSD for the area of (R, R)-sertraline under precision study was found to be within 3.0 % confirming the good precision of the method.

The %RSD for (R, R)-sertraline in intermediate precision study was within 3.5% confirming the ruggedness of the method.

2. Limit of detection and limit of quantification

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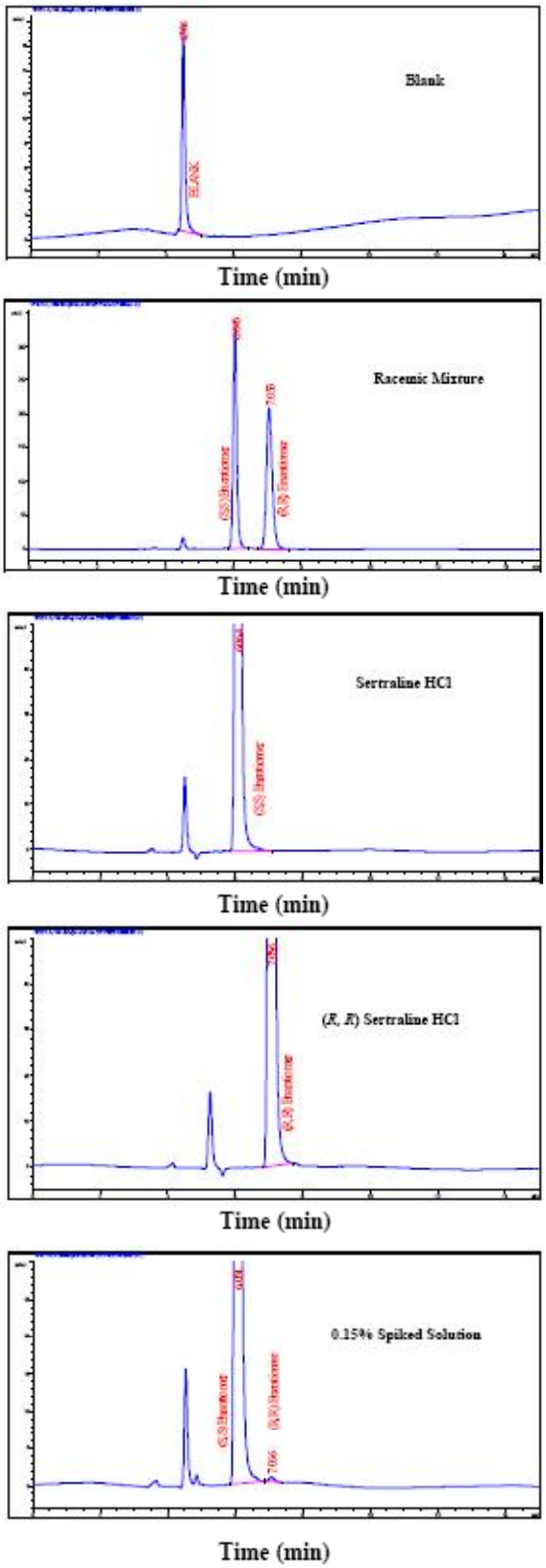

Figure 2: Typical chromatogram of blank, racemic sample, sertraline hydrochloride, (R, R) sertraline hydrochloride, spiked solution

The limit of detection for (R, R)-sertraline was 0.005µg of analyte concentration, (i.e.100µg mL^{-1}) respectively for 10µL injection volume. The limit of quantification for (R, R)-sertraline was 0.015µg of analyte concentration, (i.e. 100µg mL^{-1}) respectively for 10µL injection volume. The precision at LOQ concentration for (R, R)-sertraline was below 2.0 %.

3. Linearity

Linear calibration plot for (R, R)-sertraline was obtained over the calibration ranges tested, i.e. LOQ to 0.3 %. The correlation coefficient obtained was greater than 0.999. Slope and Y-Intercept values are 0.003 and 0.2476 respectively. Linearity was checked for (R, R)-sertraline over the same concentration ranges for three consecutive days. The percentage relative standard deviation of the slope and Y-intercept of the calibration curves for (R, R)-sertraline were 3.5 and 6, respectively. The results show that an excellent correlation existed between the peak area and concentration for (R, R)-sertraline.

4. Accuracy

The bulk sample and pharmaceutical dosage forms of sertraline hydrochloride shows the presence of (R, R)-sertraline in the level of 0.004 % and 0.002%. The percentage recovery of (R, R)-sertraline in bulk drug samples ranged from 98.3 to 101.4 (TABLE 2) and in pharmaceutical dosage forms ranged from 98.5 to 101.8 % (TABLE 3).

Robustness

In all the deliberate varied chromatographic conditions carried out (flow rate, column temperature and mobile phase composition (%DEA)), the resolution between the sertraline and (R, R)-sertraline was greater than 3.8, illustrating the robustness of the method (TABLE 4).

Solution stability and mobile phase stability

No significant changes were observed in the content of (R, R)-sertraline of sertraline hydrochloride during solution stability and mobile phase stability experiments

TABLE 2: Recovery results of (R, R)-sertraline in drug substance

Added (µg)(n=3)	Recovered (µg)	% Recovery	%R.S.D
0.075	0.074	98.3	0.7
0.150	0.15	100.1	0.4
0.225	0.228	101.4	0.8

n, Number of determinations

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TABLE 3: Recovery results of (R, R) sertraline in dosage form

Added (µg)(n=3)	Recovered (µg)	% Recovery	%R.S.D
0.075	0.074	98.5	0.8
0.150	0.151	100.8	0.5
0.225	0.229	101.8	0.7

n, Number of determinations

TABLE 4 : Results of robustness study

S.no	Parameter	Variation	Resolution between sertraline hydrochloride & (R, R)- sertraline
1	Temperature	(a) At 23°C	4.1
		(b) At 27°C	3.9
2	Flow rate	(a) At 0.6 ml/min	4.3
		(b) At 0.8 ml/min	3.8
3	%Diethylamine	(a) At -0.05%	4.0
		(b) At +0.05%	4.1

TABLE 5: Batch analysis

Bulk : batch no:	% (R, R) sertraline	Drug product batch no:	% (R, R) sertraline
SH0200305	0.004%	1728839	0.02%
SH0200309	0.004%	1728869	0.02%
SH0200311	0.004%	1728889	0.02%

when performed using the developed method. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during the study was stable up to 48 h.

CONCLUSIONS

A simple and accurate polar mode chiral HPLC method was described for the separation of sertraline hydrochloride enantiomers and quantitative determination of (R, R)-sertraline. Chiralpak-IA column was found to be specific for the sertraline hydrochloride and (R, R)-sertraline. The method was validated showing satisfactory data for all the method validation parameters tested. The developed method was robust in the separation and quantification of undesired (R, R)-enantiomer in bulk drug samples and dosage forms of sertraline hydrochloride.

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