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Separation of stereoisomers of sertraline and its related enantiomeric impurities on a dimethylated β-cyclodextrin stationary phase by HPLC

R. Nageswara Rao*, M.V.N. Kumar Talluri, Pawan K. Maurya

Analytical Chemistry Division, Discovery Laboratory, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 607, India

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ABSTRACT

A reversed-phase high-performance liquid chromatographic (HPLC) method was developed and validated for evaluating the chiral discrimination ability of CYCLOBOND I 2000 DM chiral stationary phase (CSP) towards sertraline and its related enantiomers. The effect of pH, buffer concentration as well as nature of organic modifier, flow rate and temperature on enantioselectivity was investigated. The developed reversed-phase chromatographic conditions were able to separate not only the enantiomers of sertraline but also its process related chiral impurities. The method was validated for determination of enantiomeric purity of sertraline HCl in drug substances and formulations.

1. Introduction

Depression, anxiety and obesity are some of the most common and serious health problems of the people today. Development of therapeutic agents to treat these disorders is of significant interest of recent times. Sertraline HCl or *cis*-(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride (SRT) is one of the novel drugs belonging to the group of selective serotonin reuptake inhibitors (SSRI) in brain [1]. It is useful not only in treating all types of depression but also panic disorders, social phobia, obesity, or obsessive-compulsive disorders. SRT increases the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cell, there by increases available serotonin to bind the postsynaptic receptor. The most important advantage of SRT is that it lacks the side effects of tricyclic antidepressants. The molecule of SRT contains two stereogenic centers and it is quite likely that *cis*-(1*R*,4*R*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride, *trans*-(1*S*,4*R*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride, *trans*-(1*R*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride are introduced as impurities during its synthesis. The chemical structures of the stereoisomers of sertraline and the probable process related impurities are shown in Fig. 1.

These enantiomers may have different pharmacological activities when compared to the therapeutically active molecule. Further, the USFDA imposes on the demonstration of bioavailability of chiral drugs. Thus, the development of a single enantiomer of SRT and controlling of its impurities is of great importance not only to avoid unwanted pharmaceutical and toxicological side effects but also to assure its therapeutic efficacy and safety. Thus the development of a chiral active pharmaceutical ingredient of SRT requires techniques that can quickly assess the enantiomeric purity of the drug during the development and manufacturing processes.

The most common methods employed for determination of enantiomeric purity of drugs include optical rotation, chiral HPLC and CE. Optical rotation measurements are relatively easy to perform but lack selectivity. Not only can temperature, solvent, and concentration impact the variability of the measurements but also the presence of potential impurities can negatively impact the values obtained by optical rotation [2]. Separation of enantiomers by HPLC is quite common and a variety of chiral selectors are available. Among them, cyclodextrins are very effective in LC and CE separations. Cyclodextrins form inclusion complexes with the analytes and due to difference in formation of such complexes, stereoisomers are separated. The α-, β-, γ-CD contain six, seven, and eight glucose units, respectively. The selectivity of unmodified CDs for stereoisomeric separation of analytes is limited. Cyclodextrins are

* Corresponding author. Tel.: +91 040 27193193; fax: +91 040 27160387.
E-mail addresses: rnrao55@yahoo.com, rnrao@iict.res.in, rnrao@iictnet.org (R.N. Rao).

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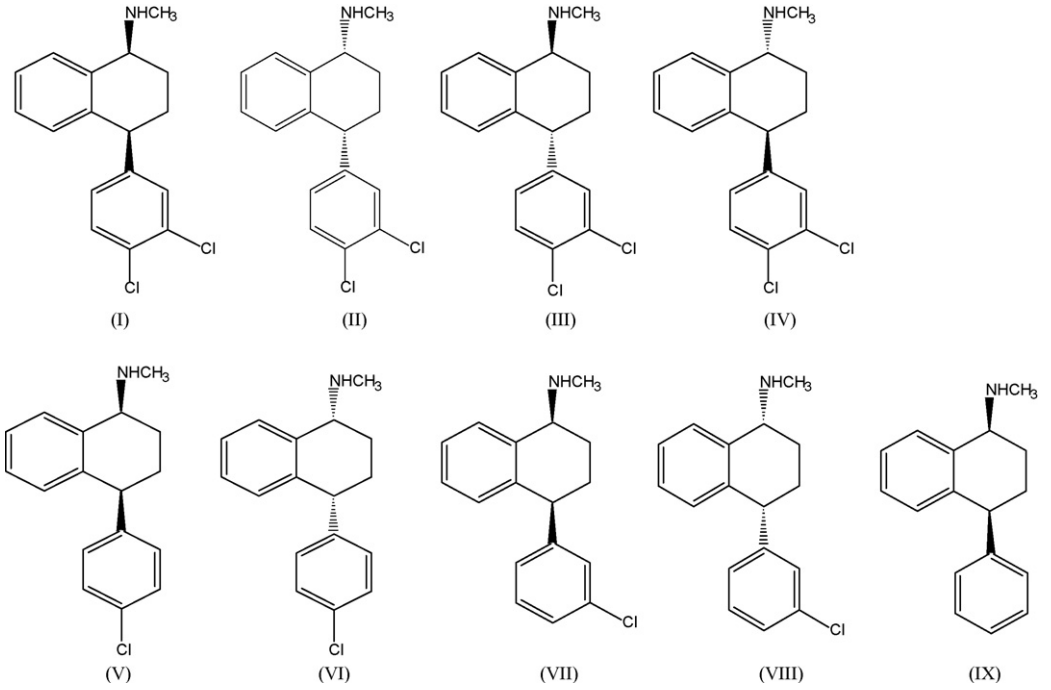

Fig. 1. Chemical structures of sertraline enantiomers [I (*Cis* 1*S* 4*S*), II (*Cis* 1*R* RS), III (*Trans* 1*S* 4*R*), IV (*Trans* 1*R* 4*S*)] and related substances [(V (4-Cl, 1*S* 4*S*), VI (4-Cl, 1*R* 4*R*), VII (3-Cl, 1*S* 4*S*), VII (3-Cl, 1*R* 4*R*), IX (1*S* 4*S*)].

generally added to the mobile phase but the disadvantages include (i) the cost of chiral additives, (ii) the complex mode of operation and (iii) inconvenient for preparative applications since the chiral additive must be removed from the enantiomeric solutes. The most common HPLC approach for resolving enantiomers involves the use of chiral stationary phases (CSPs) [3]. Various CSPs have been introduced and search for new ones continues [4].

The cyclodextrin CSPs are very important for separation of a wide range of structurally different chiral compounds [5]. These are widely used for the direct separation of drug enantiomers because of their broad applicability and the use of aqueous buffered mobile phases that are compatible with many polar compounds. The most useful and popular among them are based on β-cyclodextrin [6]. CYCOBOND I 2000 DM is a new chiral column packed with dimethyl beta-cyclodextrin which minimizes the chiral hydrogen bonding and establishes a weak dipole effect to separate structural and positional isomers in a reversed-phase system.

Literature search revealed that the determination of sertraline and its metabolites in biological fluids was thoroughly investigated. GC, GC–MS, HPLC and CE were used extensively [7–16]. The enantiomeric separation of SRT was obtained by adding CDs to the mobile phases [17]. There were a few reports on the determination of sertraline HCl and its impurities by micellar electrokinetic chromatography [18,19]. However, the constraints of narrow bore capillaries restrict the use of CE only to analytical separations. Even though, the use of CE for rapid analysis of chiral compounds is gaining acceptance, HPLC is still the preferred choice for chiral separations due to its robustness, reproducibility, detection limits and preparative separations.

The present paper describes a reversed-phase HPLC method for enantioseparation of SRT and related enantiomers using a Cyclobond column packed with dimethyl β-cyclodextrin as stationary phase. The chromatographic separations were characterized in terms of the performance parameters such as retention, selectivity and resolution. The conditions affording the best resolution were optimized and the method was validated as per ICH guidelines [20].

2. Experimental

2.1. Reagents

Sertraline HCl (*cis*-(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride) (I), *cis*-(1*R*, 4*R*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride (II), *trans*-(1*S*,4*R*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride (III), *trans*-(1*R*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride (IV), (±) *cis*-4-(4-chlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine (V, VI), (±) *cis*-4-(3-chlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine (VII, VIII), *cis*-(1*S*,4*R*)-4-phenyl-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine (IX) were procured from a local pharmaceutical firm in Hyderabad. Purified de-ionized water (Nanopure, Barnsted, USA), HPLC-grade acetonitrile, methanol, triethyl amine, trifluoroacetic acid, acetic acid (Qualigens Fine Chemicals, Mumbai, India), ammonium acetate, ammonium formate, isopropyl alcohol (S.D. Fine Chem., Mumbai, India) and absolute ethanol (Shymalakhs International, London, U.K.) were used.

2.2. Instrumentation

The HPLC system used was composed of two LC-10AT VP pumps, an SPD-10Avp diode array detector, an SIL-10AD VP auto injector, a DGU-12A degasser and SCL-10 A VP system controller (Shimadzu, Kyota, Japan). The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett Packed, Waldron, Germany) computer system.

2.3. Preparation of stock and standard solutions

Stock solutions containing 1 mg/mL of individual enantiomers of 99.8% purity of SRT and its related substances were prepared in methanol. Working standards (100 μg/mL) were prepared by dilu-

tion of individual aliquots of stock solution with the same solvent. The solutions were stable at least for 1 month at 4 °C. Appropriate dilutions of the individual working solutions of sertraline and its related substances were made and used for constructing the calibration curves.

2.4. HPLC conditions

The mobile phase was 0.4% trifluoroacetic acid–acetonitrile (80:20, v/v). The mobile phase was filtered through a Millipore membrane filter (0.2 μm) and degassed before use. The flow rate and the detection wavelength for monitoring the eluents were 0.8 mL/min and 225 nm, respectively. The analysis was carried out at 30 °C. The injection volume was 20 μL. Astec CYCLOBOND™ I 2000 DM (25 cm × 4.6 mm, 5 μm) (Supleco, PA, USA) was used for separation.

2.5. Preparation of formulation solutions

Ten tablets/capsules were powdered in a mortar and accurately weighed portion equivalent to 500 mg SRT was transferred to 100 mL volumetric flask and diluted to the mark with methanol. The solution was sonicated for 15 min, centrifuged at 3000 rpm for 10 min. Accurately measured aliquots of the supernatant were transferred to separate 5-mL volumetric flasks and diluted to 5 mL with methanol to give final concentration of 100, 300 and 500 μg/mL of sertraline. The content of each enantiomer was calculated from the peak areas in the chromatogram.

3. Results and discussion

3.1. Method development and optimization

Appropriate choice of a chiral stationary phase and mobile phase composition is very important for enantioselective separations. CDs and derivatized CDs have been used extensively, because of their stable properties, relative low price and high-column capacity. In CD-CSPs, temperature, pH, flow rate and buffer concentration influence the separation and retention times. Hydrogen bonds are associated with pH, the rate of mobile phase flow influences the interaction between the analytes and the cavity of the CDs. Hence optimization of the chromatographic conditions is necessary for enantioseparation.

Initially, 0.01 M ammonium acetate buffer (pH adjusted to 4.5 with acetic acid) with different organic modifiers, viz; methanol and acetonitrile of different compositions were tried on CHIRAL-AGP (150 mm × 4.0 mm) and CHIROBIOTEC V (150 mm × 4.6 mm) chiral columns. CHIRALCEL OD-H, OJ-H, CHIRALPACK AD-H columns (25 cm × 0.46 cm) were also tried with hexane and isopropanol or ethanol as mobile phases. These columns could not separate all the chiral pairs associated with SRT. The SRT and related substances are polar in nature and fairly soluble in methanol and water. So reverse phase conditions were tried with a Cyclobond column. The presence of steric groups near the chiral center, i.e. tetrahydro-*N*-methyl-1-naphthalenamine of analyte molecules facilitates the formation of inclusion complexes with in the cavity of dimethyl β-cyclodextrin on Cyclobond column. Small changes in the structure of sertraline could lead to large differences in enantioselectivity and provide an insight into the mechanism of chiral recognition. The chlorine substitution on the phenyl rings attached to tetrahydro-*N*-methyl-1-naphthalenamine had pronounced the effects on enantioselectivity. Probably it forms hydrogen bonding with primary alcohols at the rim of the stationary phase. The selectivity and resolution were adjusted to a limited extent by changing the organic solvent, buffer pH, and ionic strength in an isocratic mode to separate the stereoisomeric impurities.

3.1.1. Influence of the nature and concentration of uncharged organic modifiers

The retention mechanism of reversed-phase CD enantiomer separations is generally inclusion complexation. The naphthalenamine ring of the analyte gets inserted in to the hydrophobic cavity of

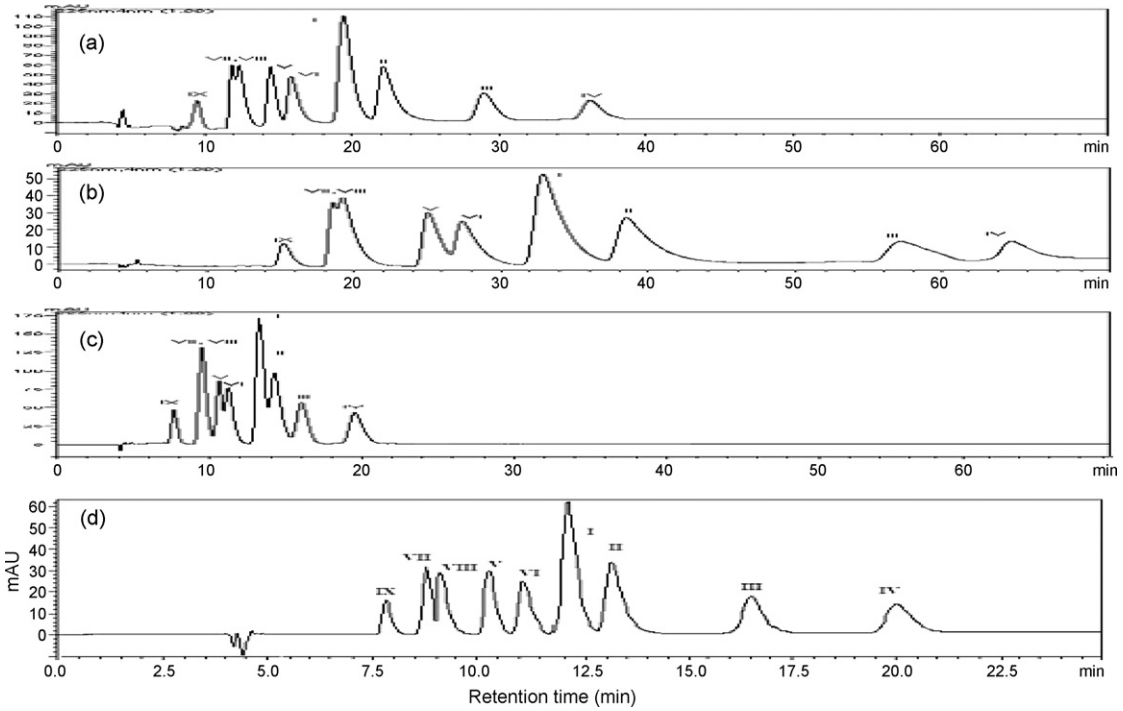

Fig. 2. Effect of different organic modifiers on separation of SRT stereoisomers and related substances. (a) EtOH (Ψ 5.2), (b) MeOH (Ψ 5.1), (c) IPA (Ψ 3.9), and (d) ACN (Ψ 5.8) (conditions: 0.4%TFA–organic modifier (80:20, v/v), pH 3.0, flow 0.8 mL/min, 30 °C).

the CD. A change in % organic modifier could be used to control retention and resolution. Decreasing the amount of polar organic modifier results in an increase in k and can affect α. Methanol, acetonitrile, isopropanol, ethanol were tried as organic modifiers. In both ethanol and methanol the resolution between VII and VIII was poor and run time was two to three times more than that of acetonitrile. In case of isopropanol, very poor resolution of enantiomer pairs or no resolution observed and the run time was similar to that of acetonitrile. Except acetonitrile, all other solvents yielded broad peaks and the separation was poor. Finally acetonitrile was used as an organic modifier for improved peak shapes, response and good resolution. The concentration of organic modifier was kept at 20% in the isocratic mode. Fig. 2a–c shows the effect of different organic modifiers on separation of chiral impurities. Fig. 2d represents the typical chromatogram of a mixture of SRT and its related substances using acetonitrile as an organic modifier.

3.1.2. Influence of mobile phase pH on retention and selectivity

pH is an important parameter with respect to separation of enantiomers on CD based CSPs, since the stability of inclusion complexes is weaker for a charged species than for a neutral species in case of a solute containing ionizable groups [21]. Retention (k) and selectivity (α) can be affected by changing the pH. The high pKa values for sertraline HCl and its related substances (8.6–9.5), suggest the operating range of pH of the mobile phase for Cyclobond column (pH 3–7). Thus, a neutral or acidic pH must be employed for separating sertraline enantiomers by Cyclobond column. The pH of the buffer (TFA) was adjusted using diluted sodium hydroxide from 3 to 7. The selectivity (α) was slightly increased from pH 3–7. There was a gradual increase in retention of the compounds, from 3 to 5 pH, a slight decrease at pH 6 and increase at pH 7 were observed. According to acid–base equilibria, as pH decreases, bases gain a proton (ionize), becoming more hydrophilic, and less retained by reverse phase separations. Therefore, the retention was relatively low under low pH values for basic analytes. There was a transition range between low to high pH, in which the retention depends on the ionization state or degree of ionization of the analyte [22]. Armstrong reported the effect of pH on retention and selectivity in reversed-phase liquid chromatographic separation of different basic analytes on cyclodextrin bonded phases. As the pH increases, three different types of behavior were observed. Where solute retention goes up from pH 4.1 to 5.5 and then decreases to less than the original at pH 7.0. The protonated species do not complex strongly with the β-CD support [23] and the degree of ionization, analyte structure, i.e., position, size, hydrogen bonding functionalities, and degree of solvation, including any secondary interactions affect the basic analyte retention [24]. The tailing of peaks was observed to be less at pH 3.0 and increased with a raise in pH. Resolution of enantiomeric pairs decreased with the increase of pH. Thus pH 3.0 was found to be suitable with required resolution (Rs) and less tailing (As) for most of the enantiomers. The selectivity, retention, resolution and tailing of all chiral compounds on Cyclobond column are shown in Fig. 3.

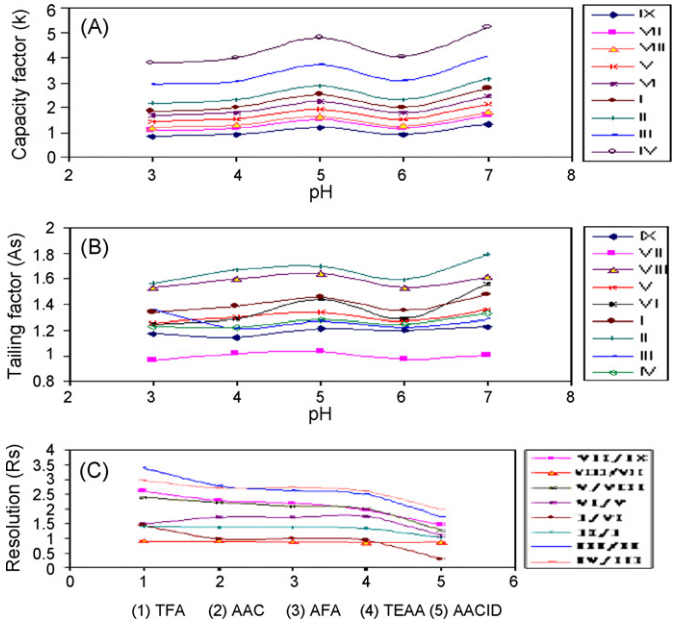

Fig. 3. Effect of pH on (A) capacity factor (k') and (B) tailing factor (As) and different buffers on (C) resolution (Rs).

Table 1
The system suitability data of SRT and its impurities.

Compound	Retention time (min)	Separation factor (α)	Resolution (Rs)	Tailing factor (As)
I	12.10	1.12	1.45	1.33
II	13.32	1.15	1.53	1.56
III	16.43	1.34	3.29	1.34
IV	20.23	1.31	3.30	1.22
V	10.25	1.20	1.79	1.25
VI	11.25	1.16	1.62	1.24
VII	8.79	1.27	1.96	0.96
VIII	9.23	1.09	0.95	1.52
IX	7.81	1.27	–	1.17

3.1.3. Effect of the buffer and its concentration

Since buffers can easily get included into the CD cavity, the choice of a suitable buffer is very important. It improves not only efficiency but also reproducibility of CD based separations. The effect of type of buffer on separation was studied on Cyclobond column maintained at 30 °C. Initially, 10 mM of ammonium acetate (AAc), ammonium formate (AFA), 0.1% (v/v) of acetic acid (AACID), triethylammonium acetate (TEAA) and trifluoroacetic acid (TFA) were used to separate the stereo isomeric compounds. Resolution was high for almost all the compounds in TFA, than other four buffers under study. Fig. 3C shows the effect of buffer type on resolution. As the buffer concentration increased, peaks became sharper and resolutions were increased. Tailing was decreased with an increase in concentration of TFA. The desired symmetry and resolution were obtained with 0.4% TFA and it was used for further optimization of other variables.

3.1.4. Effect of flow rate and column temperature

The effect of flow rate and temperature of the column on the retention and resolution of the compounds I–IX was investigated in the range from 0.4 to 1.2 mL/min at 20, 30 and 40 °C. The reten-

Table 2
Accuracy of the determination of sertraline HCl in formulations.

Compound	Concentration (mg mL^{-1})		SD[a]	RSD (%)	Recovery (%)
	Taken	Found			
Sertraline HCl	0.050	0.049	0.049	0.83	99.33
	0.100	0.098	0.002	2.04	98.00
	0.300	0.299	0.010	3.59	99.88
	0.400	0.399	0.010	2.69	99.91
	0.500	0.491	0.023	4.80	98.26

[a] Average of six determinations (n = 6).

Table 3
Results of analysis of bulk drugs and formulations by HPLC.

Sample	Impurities (%, w/w)								Assay (%, w/w)	RSD (%)
	II	III	IV	V	VI	VII	VIII	IX	I	
BD1	0.02	–	0.01	–	0.03	–	0.01	–	99.81	0.02
BD2	0.01	0.01	–	0.02	–	–	–	0.01	99.95	0.06
TAB1	0.04	0.01	–	0.02	–	–	0.01	0.01	99.42	0.15
TAB2	0.02	–	0.02	0.03	–	–	–	0.01	99.58	0.10
CAP1	0.04	–	0.01	0.01	0.02	–	0.01	–	99.25	0.36
CAP2	0.04	0.02	–	–	0.01	–	0.02	–	97.98	2.04

tion factor (k'), selectivity (α) and resolution (Rs) were measured. It was observed that the flow rate and temperature exhibited a strong influence on Rs. The k' values decreased when the column temperature was increased. The observed changes in k' values could be probably due to the decrease in the individual bond energies for each enantiomer with increasing column temperature. In all cases, a significant decrease of Rs was observed with the increase of flow rate and temperature. The decrease in selectivity (α) was also significant with increase of temperature but not so in case of increase in the flow rate. The observed decrease in Rs was associated to the reduction in the retention times due to decrease in interactions between the stationary phase and the enantiomers of the chiral molecules. In the low range of flow rate and temperatures, the retention times were higher leading to higher interaction times among the stationary phase and the individual molecules. These effects increase the power of column discrimination. The required resolution (Rs) had reached at a flow rate of 0.8 mL/min at 30 °C. It was observed that as the number of chlorine atoms increases in the analyte molecules the retention by Cyclobond column was more. The para substituted chlorine analytes retained more than meta chloro analytes while unsubstituted analytes retained less by the column.

3.2. Validation

The developed method was validated for system suitability, accuracy, linearity, limit of detection, limit of quantification, repeatability, intermediate precision, and ruggedness. The results demonstrated that the method was suitable for determination of the potency and enantiomeric purity of *SS*, *RR*, *SR*, *RS*-enantiomers of SRT in the presence of potential impurities in drug substances and products.

3.2.1. System suitability

Performance of the method was determined by injecting a mixture containing 0.1 mg/mL of *cis*-SRT (*SS*, *RR*) and *trans*-SRT (*SR*, *RS*) enantiomer. These enantiomers form a critical band pair in the chromatogram. Therefore qualification criteria were set as a resolution between the two enantiomers 1.5 and tailing factor 1.2, to ensure baseline separation and symmetrical peaks. The data are recorded in Table 1.

3.2.2. Accuracy

Accuracy of the assay of sertraline HCl was investigated. Samples of known concentrations were analyzed and compared with the

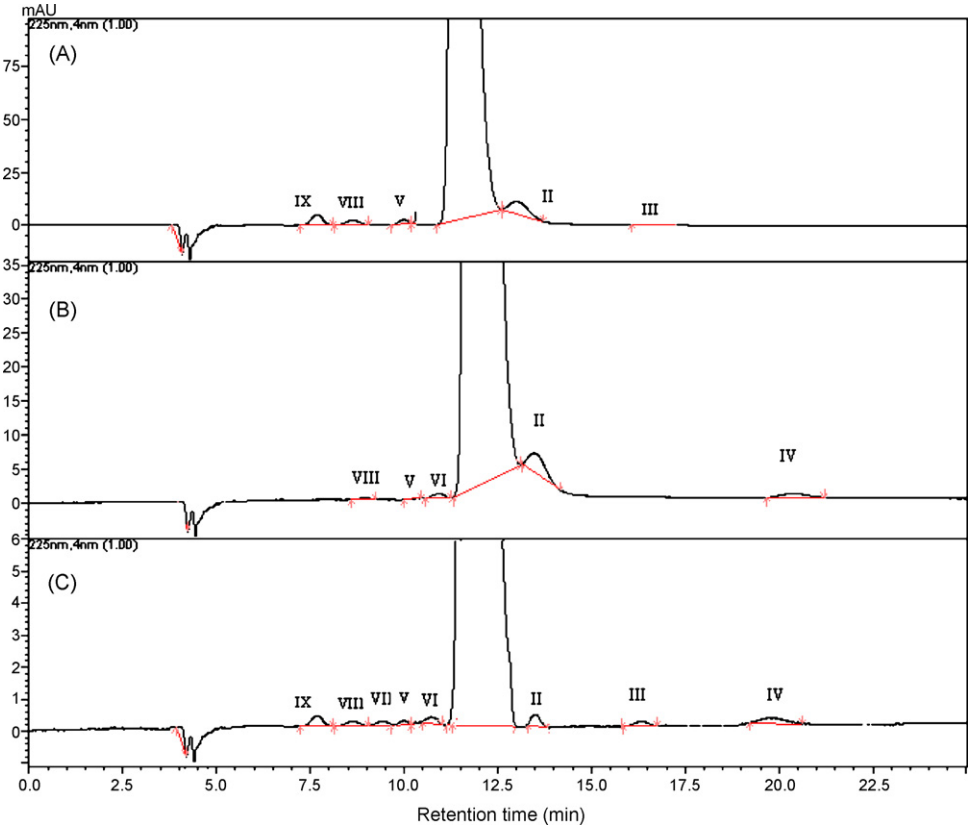

Fig. 4. HPLC chromatograms of (A) TAB1, (B) CAP1, (C) bulk drug spiked with 0.1% impurities.

true values. The accuracy study was performed with five concentrations of sertraline hydrochloride at 0.05, 0.1, 0.3, 0.4, and 0.5 mg/mL analyzing seven solutions for each concentration. Table 2 shows the mean recovery per each concentration (98.26–99.91%).

3.2.3. Precision

The precision (repeatability) of the chromatographic procedure was assessed by analyzing seven solutions for three chosen concentrations from the calibration plots of each substance. The results showed that the repeatability of the system was satisfactory.

3.2.4. Linearity

Linearity of sertraline HCl was found in the range of 0.001–1.000 mg/mL. *RR*, *SR*, *RS*-enantiomers and other chiral impurities of 1–10 μg/mL were determined. The data were subjected to statistical analysis using a linear-regression model, the slope and intercept were calculated. The results indicated a good linearity with correlation coefficients (r^2) in the range of 0.997–0.999.

3.2.5. LOD and LOQ

Limit of detection (LOD) was measured as the lowest amount of the analyte that could be detected to produce a significant response. It was approved by calculations based on the standard deviation of the response (σ) and the slope (S) of the calibration curve at the levels approaching the limits according to equation LOD 3.3 (σ/S) [20]. LOD for sertraline hydrochloride was 0.05 μg/mL and for its impurities (II–IX) 0.02, 0.1, 0.13, 0.24, 0.18, 0.15, 0.22 and 0.16 μg/mL, respectively. Limit of quantification (LOQ) was measured as the lowest amount of analyte that can be reproducibly quantified above the baseline noise, for which duplicated injections resulted in a RSD ≤ 2%. A practical LOQ giving a good precision and acceptable accuracy was 0.18 μg/mL for sertraline HCl and 0.08, 0.35, 0.45, 0.72, 0.60, 0.53, 0.73 and 0.53 μg/mL for its impurities II–IX, respectively.

3.2.6. Ruggedness

Ruggedness of the method was determined by performing assay and quantification of SRT-enantiomer on two different HPLC systems and columns by two analysts. The method was found to be rugged as assay values of SRT-enantiomer did not vary significantly with overall relative standard deviation <1%.

3.3. Analysis of real samples

Different batches of sertraline HCl bulk drugs, tablets and capsules were analyzed and the results are recorded in Table 3. The peaks were identified by injecting and comparing with the retention times of the individual compounds. The contents of impurities relative to sertraline were in the range of 0.01–0.05%. Fig. 4 shows the typical chromatograms for identification of SRT and its related substances in formulations. The assay of sertraline was carried out by diluting the above solutions to 50–500 μg/mL with the mobile phase.

4. Conclusions

The proposed RP-HPLC method allowed not only separation of *cis* (1*S* 4*S*), (1*R* 4*R*) but also *trans* (1*S* 4*R*), (1*R* 4*S*) enantiomers of sertraline HCl along with 5 other related enantiomers due to high selectivity of the chromatographic system. The Cyclobond I 2000 DM was found to be the most effective cyclodextrin-based CSPs for separating the enantiomers of sertraline and its related enantiomers in a reverse phase mode. The elution sequence: without chlorine substitution (IX) > meta chloro (VII, VIII) > para chloro (V, VI) > dichloro *cis* (I, II) > dichloro *trans* (III, IV). The chromatographic conditions were optimized by studying the effects of temperature of the column and concentration and pH of TFA buffer. The developed method was found to be selective, sensitive, precise, linear, accurate and reproducible in determining the sertraline and its potential impurities, which may be present at trace level in the finished products. The method could be used in the quality control and purity testing of sertraline as it was sensitive, precise and accurate.

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