SYNTHESIS, CHARACTERIZATION, IDENTIFICATION AND QUANTIFICATION OF SERTRALINE HYDROCHLORIDE RELATED IMPURITIES

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ABSTRACT
Sertraline hydrochloride is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class, is synthesized for commercial use as a drug substance in highly pure form. During the synthetic process development studies of Sertraline hydrochloride, an impurity was detected in the final product at levels ranging from 0.05% to 0.15% in gas chromatography (GC) method and its molecular weight was determined by LC–MS analysis. The impurity was identified as 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-ol impurity, synthesized and characterized, the process of its formation was discussed in detail. After qualification of impurity standard, it was used as a reference standard for development of quantification by high performance liquid chromatography (HPLC) method.

HIGHLIGHTS
- Unknown impurity of sertraline hydrochloride is synthesized and isolated by preparative HPLC.
- Impurity is identified by LC-MS and NMR techniques.
- Reverse phase HPLC method is developed for quantification of specified impurity in sertraline hydrochloride.
- Chiral HPLC method is developed for further separation of the enantiomer of the impurity.

KEY WORDS: Sertraline hydrochloride, method development, isolation, characterization, LC-MS, NMR, HPLC.

INTRODUCTION
Sertraline is chemically known as (15,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine. Sertraline, having a chemical structure as shown in figure 1, has a molecular weight of 306.229 and its molecular formula is C17H17Cl2N.

Sertraline (trade names Zoloft and others) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It was introduced to the market by Pfizer in 1991. Sertraline is primarily prescribed for major depressive disorder in adult outpatients as well as obsessive-compulsive disorder, panic disorder, and social anxiety disorder, in both adults and children. In 2013, it was the most prescribed antidepressant and second most prescribed psychiatric medication (after alprazolam) on the U.S. retail market, with over 41 million prescriptions.

Sertraline hydrochloride drug substance is official in United State pharmacopoeia (USP) as well as European Pharmacopoeia (EP). The listed organic impurities and method of analysis by gas chromatography is same in both pharmacopoeias. In most of the commercial batches one unknown impurity is observed at relative retention time (RRT) 0.66 is about 0.05% to 0.15% (figure 2). Here in this research article attempts were made to synthesize, isolate, characterize, identify and quantify the unknown impurity of RRT 0.66.

MATERIAL AND METHOD
1.0 Manufacturing Process of Sertraline Unknown Impurity at RRT 0.66
1.1 Stage-1: Preparation of 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one
1.1.1 Reaction Scheme

\[
\text{α-Naphthol} + \text{Chlorobenzene} \rightarrow \text{4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one}
\]

1.1.2 Raw Materials

| Sr. No. | Raw Material     | Qty. | Unit | Mol. Wt.  | Moles | Molar Ratio |
|---------|------------------|------|------|-----------|-------|-------------|
| 1       | α-Naphthol       | 50   | gm   | 144.17    | 0.346 | 1           |
| 2       | Chlorobenzene    | 275  | ml   | 112.56    |       | 5.5 T       |
| 3       | Aluminium chloride | 100  | gm   | 133.34    | 0.74  | 2.2         |
| 4       | Water            | 500  | ml   | 18        |       | 10 T        |

1.1.3 Process

1. In a 500 ml four neck RBF in oil bath with reflux condenser, charge α-Naphthol (50 gm).
2. Add Chlorobenzene (175 ml) and start stirring.
3. Charge Aluminium chloride (100 gm) lot wise at 25-30°C in 30 min.
4. After complete addition of aluminium chloride, start heating to raise the temperature of reaction mass to 60-65°C.
5. Maintain the temperature of the reaction mass to 60-65°C and check the progress of the reaction on TLC till complete conversion of α-Naphthol (Limit: 1.0%). If not, then maintain further the reaction mass to 60-65°C till α-Naphthol is in limit.
6. After completion of reaction, cool the reaction mass to 30-35°C.
7. Add the reaction mass slowly into ice-cold water (500 ml) with maintain temperature to 10-15°C.
8. Separate out the organic and aqueous layer; wash aqueous layer with chlorobenzene (50 ml x 2 times) and send aqueous layer to ETP as waste.

9. Collect all organic layers and distill out chlorobenzene under vacuum at 70-75°C completely to get oily product. This product is mixture of 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one and 4-(2-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one

\[
\text{Observed Yield} = 58 \text{ gm} \\
\text{Theoretical Yield} = 88.75 \text{ gm} \\
\text{Percentage Yield} = 65.35 \%
\]

10. This two products are separated by column chromatography [Silica bed (60-120 mesh) and mobile phase used is Ethylacetate : Hexane = 2 : 8]

\[
\text{A} = 4-(4\text{-chlorophenyl})-3,4\text{-dihydronaphthalen-1(2H)-one} \\
\text{B} = 4-(2\text{-chlorophenyl})-3,4\text{-dihydronaphthalen-1(2H)-one}
\]

11. Separate out 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one with requisite amount and proceed further for next reduction step.

The experimental data is as follows:

| Sr. No | Batch Number | Input | Output | Reaction condition | Result |
|--------|--------------|-------|--------|--------------------|--------|
| 1      | SET/40/I/270/45-B | 17 gm | 2.5 gm | Column purification | Desired Unknown = 99.39% |
| 2      | SET/40/I/270/48 | 15 gm | 3 gm   | Column purification | Desired Unknown = 98.19% |

1.2 Stage-II: Preparation of 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-ol

1.2.1 Reaction Scheme

\[
\text{4-(4-chlorophenyl)-3,4-dihydro-naphthalen-1(2H)-one} \rightarrow \text{4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-ol naphthalen-1(2H)-one}
\]
1.2.2 Raw Materials

| Sr. No. | Raw Material                                      | Qty. | Unit | Mol. Wt. | Moles | Molar Ratio |
|---------|---------------------------------------------------|------|------|----------|-------|-------------|
| 1       | 4-(4-chlorophenyl)3,4-dihydronaphthalen-1(2H)-one | 3    | gm   | 256.72   | 0.0116| 1           |
| 2       | Methanol                                          | 50   | ml   | --       | --    | --          |
| 3       | NaBH₄                                             | 0.5  | gm   | 37.83    | 0.0132| 1.14        |
| 4       | MDC                                               | 150  | ml   | --       | --    | --          |

1.2.3 Process
1. Arrange 250 ml four neck RBF in water bath and take methanol (50 ml) into it.
2. Add 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one (3.0 gm) and start stirring.
3. Cool the reaction mass to 10-15°C.
4. Add NaBH₄ (0.5 gm) to the reaction mass under stirring with maintaining temperature of the reaction mass at 10-15°C.
5. Maintain the reaction mass 2 hr at 10-15°C and check for the complete conversion of 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-one on TLC (Limit: NMT1.0%). If not, then add NaBH₄ (0.1 gm) and check for completion.
6. After completion of reaction, distill out methanol from the reaction mass completely.
7. Add water (50 ml) and stir the reaction mass at 30-35°C.
8. Extract the compound with MDC (50 ml x 3 Times) and separate the organic MDC layer.
9. Collect all organic MDC layer and wash with water (25 ml). Separate out the organic layer and distill out MDC completely at 40-45°C.
10. Apply vacuum to remove MDC completely to get oil as 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-ol.

Observe Yield = 2.6 gm
Theoretical Yield = 3.02 gm
Percentage Yield = 86.09%

11. This product is a mixture of following isomers

![Isomers Diagram]

The experimental data is as follows

| Sr. No | Batch Number | Input | Output | Reaction condition | Result          |
|--------|--------------|-------|--------|--------------------|-----------------|
| 1      | SET/40/II/270/46 | 1.2 gm | 0.8 gm | Reduction in NaBH₄ | RT-11.98 = 51.15 %, RT-12.33 = 47.58 % |
| 2      | SET/40/II/270/49 | 3 gm   | 3 gm   | Reduction in NaBH₄ | RT-11.99 = 50.59 %, RT-12.33 = 49.23 % |

1.3 Characterization Data

1.3.1 ¹H-NMR Spectra
Instrument: Bruker NMR with 400 MHz
Solvent: DMSO-d₆
### Interpretation Data

| Chemical Shift Observed (ppm) | Details          | Protons                        |
|-------------------------------|------------------|--------------------------------|
| δ 1.7078 - δ 1.8670           | Multiplet, 2H    | -CH₂- group protons (f)*       |
| δ 1.9724 - δ 2.0898           | Multiplet, 2H    | -CH₂- group protons (g)*       |
| δ 4.4735 - δ 4.5746           | Triplet, 1H      | >CH₃- group proton (e)*        |
| δ 4.6395 - δ 4.7354           | Multiplet, 1H    | >CH₃- group proton (h)*        |
| δ 5.2692 - δ 5.2887           | Singlet, 1H      | -OH group proton (i) (D₂O-Exchangeable)* |
| δ 6.6400 - δ 7.5387           | Multiplet, 8H    | Aromatic proton (a, b, d, j, k, l, m)* |
| Total                         | 15 Protons       |                                |

*Note: All the Protons counts are observed in double due to the presence of diastereomers.

#### 1.3.2 ¹³C-NMR Spectra

**Instrument:** Bruker NMR with 400 MHz  
**Solvent:** DMSO-d₆

### Interpretation Data

| Chemical Shift Observed (ppm) | Carbons         |
|-------------------------------|-----------------|
| 26.51-27.46                   | C-14*           |
| 29.78-31.03                   | C-15*           |
| 41.47-42.07                   | C-13*           |
| 66.69-66.95                   | C-16*           |
| 126.59-126.70                 | C-7*            |
| 127.37-127.71                 | C-10*           |
| 128.30-128.35                 | C-8*            |
| 128.43                        | C-9             |
| 129.10-129.31                 | C-5 and C-3     |
| 129.63-129.85                 | C-2             |
| 131.06-131.32                 | C-6             |
| 133.33                        | C-4             |
| 138.24-138.36                 | C-11            |
| 141.32-141.88                 | C-1             |
| 144.09-144.34                 | C-12            |
| Total                         | 16 Carbons      |

*Note: The presence of two Carbon Signals for each of the carbon indicates the presence of diastereomers.

#### 1.3.3 IR Spectra

**Instrument:** Perkin Elmer

| Functional group | Characteristic absorption(s) cm⁻¹ |
|------------------|----------------------------------|
| -C-OH stretch    | 3377.74 cm⁻¹                     |
| Ar-Cl stretch     | 738.93 cm⁻¹                      |

#### 1.3.4 Mass Spectra

**Instrument**

Molecular ion peak observed as (M+H) = **258.0, 259.1, 260.1**

The above characterization (¹H and ¹³C NMR) and GC purity study was carried out using crude material batch No. SET/40/II/270/49. On basis of above study, it can be concluded that the unknown impurity at RRT=0.66 may be a mixture of four isomers [(R,R), (S,S), (R,S) and (S,R)] of 4-(4-chlorophenyl)-3,4-dihydronaphthalen-1(2H)-ol.

In GC purity of crude material batch No. SET/40/II/270/49 split peak was observed at RRT 0.66 so further study is carried out for separation and isolation of two peaks by HPLC. After few trials both peaks were separated by HPLC in batch No. SET/40/II/270/49. The first peak is eluting at retention time 27.7 minutes of purity 45.69% and second peak at 32.9 minutes of purity 54.19%. The method was developed suitable for liquid chromatography-mass spectrometry (LCMS) study and mass of both peaks are determined. The mass of both peaks is observed same.

#### 2.0 LCMS Spectra

**Instrument:** LCMSD_ALS  
LCMS chromatogram and spectrum is attached in figure 3.

Molecular ion peak observed in LCMS at RT=13.02 min is 257.92.

Molecular ion peak observed in LCMS at RT=14.3 min is 257.76.
Further for isolation of both peaks of batch No. SET/40/II/270/49 preparative HPLC Technique was used.

After isolating both peaks HPLC purity of individual isomer was checked and found Isomer-I eluting at retention time 24.5 minutes purity is 98.29% and peak purity on PDA detector is passing. Second isomer eluting at retention time 28.9 minutes purity is 99.18% and peak purity on PDA detector is passing.

Isomer-I and Isomer-II both are separately spiked in the sertraline hydrochloride sample and injected in GC purity method. It is found that the area of unknown impurity at RRT 0.66 has been increased in the sample spiked with Isomer-II. Isomer-II is exactly matching with the unknown impurity of RRT 0.66. While Isomer-I is eluting closely after Isomer-II i.e. at RRT 0.67. For further confirmation GCMS was carried out and found that fragmentation pattern of unknown impurity at RRT 0.66 and Isomer-II is same.

From the above study it can be concluded that the unknown impurity at RRT 0.66 in GC purity is Isomer-II.

For quantification of unknown impurity at RRT 0.66 that is Isomer-II by GC the low load of Isomer-II i.e. 0.1% with respect to test concentration was injected in GC but it was found that the pure Isomer-II is thermally unstable and after degradation one degradant peak is eluting at retention time 7.1 minutes.

From above study it is decided that unknown impurity in GC of RRT 0.66 to be disregarded in GC purity method and it has to be quantified by following HPLC method.

3.0 REVERSE PHASE HPLC METHOD OF ANALYSIS FOR QUANTIFICATION OF ISOMER I AND ISOMER II OF 4-(4-CHLOROPHENYL)-3,4-DIHYDRONAPHTHALEN-1(2H)-OL

3.1 Instrumentation and liquid chromatographic conditions: HPLC method was performed using a Shimadzu-2010 HPLC system with UV detector and Lab Solution software. Separation was achieved with the mixture of Mobile Phase-A: Water:Acetonitrile:Methanol (500:450:50 v/v/v) and mobile phase-B: acetonitrile in gradient elution with timed programme T0min/A:B: T0/100:00; T0max/100:00; T0/10:90; T0/10:90; T0/10:00 and T0/10:00 with flow rate 1.0 mL/min. The column temperature was maintained at 30°C. Ultraviolet detection was performed at 210 nm. Injection volume is 10 μL and run time is 70.0 minutes. HPLC column is Cosmosil C18, 250 mm length, 4.6 mm internal diameter and 5μm particle size.

3.2 Preparation of standard and sample solutions
Diluent: Acetonitrile : Water (50:50 v/v)

3.2.1 Blank preparation: Same as diluent.

3.2.2 Standard stock solution: Weigh and transfer accurately about 3.0 mg each of Isomer-I and Isomer-II Reference Standard in to 20 mL volumetric flask, add diluent , sonicate to dissolve and make up to the mark with diluent.

3.2.3 Standard solution: Transfer 1.0 mL Standard stock solution to 100 mL volumetric flask and diluted up to mark with diluent.

3.2.4 Test solution: Weigh and transfer accurately about 5.0 mg of sample in to 5mL volumetric flask, add diluent , sonicate to dissolve and make up to the mark with diluent.

3.3 Procedure: Inject blank (diluent), Standard solution and Test solution in the chromatograph. The retention time of Isomer-I is about 24.5 minutes and Isomer-II is about 28.9 (figure 4 to 6).

3.3.1 System Suitability Criteria
The system is suitable for analysis, if and only if,
a) Resolution between Isomer-I and Isomer-II peak should be Not less than 3.0
b) % RSD for areas of Isomer-I and Isomer-II in six replicate injections of standard solution should be not more than 5.0.

3.4 CALCULATION
Integrate the peaks of Isomer-I and Isomer-II in standard and test solution and calculate both isomers by following formula.

% specified impurity =
Area of isomer I or isomer II peak in Test solution x Conc of specified isomer in std. solution (mg/mL) x P x 100
Avg. area of isomer I or isomer II peak in std. solution x Conc of test solution (mg/mL) x 100

Where, P=Potency of specified isomer I or isomer II reference standard.

4.0 CHIRAL HPLC METHOD OF SEPARATION OF ENANTIOMERS OF ISOMER I AND ISOMER II OF 4-(4-CHLOROPHENYL)-3,4-DIHYDRONAPHTHALEN-1(2H)-OL

4.1 Instrumentation and liquid chromatographic conditions
HPLC method was performed using a Shimadzu-2010 HPLC system with UV detector and Lab Solution software. Separation was achieved with the mixture of Mobile Phase- n-Hexane:Isopropyl alcohol:1-Propanol:Diethyamine(490:0.5:2.5:0.5 v/v/v/v) in isocratic elution. The column temperature was maintained at 25°C. Ultraviolet detection was performed at 275 nm. Injection volume is 20 μL and run time is
60.0 minutes. HPLC column is Chiralpak AD-H, 250mm length, 4.6 mm internal diameter and 5µm particle size.

4.2 Preparation of standard and sample solutions
Diluent: Same as mobile phase.

4.2.1 Blank preparation
Same as diluent.

4.2.2 System suitability solution
Weigh and transfer accurately about 2.0 mg each of Isomer-I and Isomer-II Reference Standard into 20 mL volumetric flask, add diluent, sonicate to dissolve and make up to the mark with diluent.

4.2.3 Test solution
Weigh and transfer accurately about 5.0 mg of sample into 5mL volumetric flask, add diluent, sonicate to dissolve and make up to the mark with diluent.

4.3 Procedure
Inject blank (diluent), system suitability solution and Test solution in the chromatograph. The retention time of Isomer-I peak-I is about 26.83 minutes, Isomer-II peak-I is about 31.24 min, Isomer-II peak-II is about 34.75 min and Isomer-I peak-2 is about 41.94 min (figure 7 to 9).

4.3.1 System Suitability Criteria
The system is suitable for analysis, if and only if,

a) Resolution between closely eluting Isomer-II peak-1 and Isomer-II peak-2 should be Not less than 1.5 in system suitability solution.

RESULTS AND DISCUSSIONS

Figure-1-Sertraline hydrochloride structure

Figure-2-Sertraline hydrochloride commercial batch GC purity test solution chromatogram with unknown impurity at RRT 0.66 (retention time 11.834 minutes)

Figure-3-LCMS chromatogram and spectrum-isomer I at RT 13.02minutes mass is 257.92 and isomer II at RT 14.30minutes mass is 257.76

Figure-4-Blank chromatogram of RP-HPLC method for quantification of isomer I and isomer II
II peak-II is about 34.75 min and Isomer-I peak-2 is about 41.94 minutes.

Organic impurities of Sertraline hydrochloride is determined by gas chromatography method as per USP and EP monograph. In most of the commercial batches one major unknown impurity was observed at RRT 0.66 consistently about 0.15%. This impurity is synthesized with the help of chemical synthesis, by thin layer chromatography and by preparative HPLC.

During chemical synthesis it had been observed that two impurities are formed which are eluting very closely in GC purity method at RRT 0.66 and 0.67. This chemically synthesized crude material with mixture of two impurities was applied on preparative HPLC and two separate pure impurities are isolated. The purity of isomer I is 98.29% and isomer II is 99.18%. Characterization of Isomer I and isomer II is done for structure elucidation with infra-red spectrometry (IR), NMR and mass.

CHARACTERIZATION OF ISOLATED ISOMER I

Accordingly a first aspect of the present invention provides a compound Isomer I having chemical name (1S,4S)-4-(4-chlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-ol and (1R,4R)-4-(4-chlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-ol

\[
\begin{align*}
\text{ISOMER I: MS (ESI positive ion) m/z 258.0 (M + 1)}
\end{align*}
\]

\[
\begin{align*}
^1\text{H-NMR (DMSO, 400 MHz):} & \quad \delta 1.68-1.89 (m, 4H), 1.99-2.51 (m, 4H), 4.47-4.57 (t, 2H), 4.64-4.65 (m, 2H), 5.27 and 6.54 (broad singlets, D}_2\text{O Exchangeable, 2H)}
\end{align*}
\]
6.64-6.68 (dd, 2H), 6.81-6.90 (dd, 2H), 7.08-7.13 (dd, 2H), 7.19-7.46 (m, 6H), 7.47-7.54 (m, 4H)

\(^{13}\)C-NMR (DMSO, 400 MHz): 26.50, 27.45, 29.77, 31.03, 41.46, 42.06, 66.69, 66.94, 126.59, 126.70, 127.38, 127.58, 127.72, 128.31, 128.37, 128.43, 129.10, 129.23, 129.32, 129.64, 130.06, 131.32, 133.33, 138.24, 138.36, 141.33, 141.88, 144.09, 144.33.

CHARACTERIZATION OF ISOLATED ISOMER II

Accordingly a second aspect of the present invention provides a compound Isomer II having chemical name (15R,4R)-4-(4-chlorophenyl)-1,2,3,4-tetrahydrobenzophenanthrene-1-ol and (1R,4S)-4-(4-chlorophenyl)-1,2,3,4-tetrahydrobenzophenanthrene-1-ol.

CONCLUSION

In conclusion, a process related impurity of sertraline hydrochloride produced according to the synthetic route explained above. Two impurities were identified, synthesized and characterized. Structural elucidations of both synthesized compounds were done by using proton NMR, IR and MS spectral data. Thus, the regulatory requirement was fulfilled by characterizing this impurity and the prepared impurity standard was used during analytical method development studies. Hence, the reported route of synthesis can be used for isolation of impurity and the analytical methods can be used for routine determination of both impurities in sertraline hydrochloride in quality control laboratories in the pharmaceutical industry.

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