Quantification of Genotoxic Impurities in Key Starting Material of Sertraline Hydrochloride by Simple and Sensitive Liquid Chromatography Technique

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Abstract: Background: Schiff base is an advanced key starting material of Sertraline hydrochloride. Schiff base is synthesized using two raw materials 1-Naphthol and 1,2-Dichlorobenzene which are potentially genotoxic impurities.

Objective: Genotoxic impurities need to be controlled in key starting material to avoid carry forward in the active pharmaceutical ingredient. For trace level quantification of impurities a sensitive, accurate and cost effective method is developed by simultaneous estimation of both impurities.

Methods: Reverse phase high performance liquid chromatography (HPLC) method was developed and validated for determination of both impurities in Schiff base. HPLC column Cosmosil MS-II C18, 100 mm X 4.6 mm, 3 µm particle size with ultra-violet detector (UV) was used.

Results: The calibration curve of 1-Naphthol and 1,2-Dichlorobenzene showed good linearity over the concentration range of 0.25 μg/g to 7.5 μg/g and 1.5 μg/g to 7.5 μg/g and the regression coefficient was 0.999 and 0.998 respectively. Method had very low limit of detection (LOD) and limit of quantification (LOQ) of both analytes which proves that the method is sensitive and suitable for quantification of compounds at trace level.

Conclusion: The proposed method is specific, linear, accurate, rugged and precise. Genotoxic impurities 1-Naphthol and 1,2-Dichlorobenzene are quantified and controlled in the key starting material of Sertraline hydrochloride. The validated method can be used in quality control unit in pharmaceutical industry.

Keywords: Sertraline, genotoxic, impurity, method development, validation, liquid chromatography.

1. INTRODUCTION

The chemical name of Sertraline is (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydro-1-naphthalen-1-amine. The molecular formula is C₁₇H₁₇Cl₂N and molecular weight 306.229. Refer chemical structure given in Fig. (1). Sertraline is an antidepressant of the selective serotonin re-uptake inhibitor (SSRI) class [1, 2]. It is used in obsessive-compulsive disorder, panic disorder, and social anxiety disorder, in both adults and children [3-5]. In 2013, it was the most prescribed antidepressant and second most prescribed psychiatric medication (after alprazolam) in the U.S. retail market, with over 41 million prescriptions [6]. Sertraline hydrochloride drug substance monograph is official in the United State pharmacopeia (USP) [7] as well as European Pharmacopoeia (EP) [8].

Schiff base is an advanced intermediate used for the synthesis of Sertraline hydrochloride. The chemical name of Schiff base is N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthaleneimine. It is commercially manufactured using two raw materials 1-Naphthol and 1,2-Dichlorobenzene (ODCB) [9]. Both compounds are reported as potential genotoxic impurities. Dichlorobenzenes are reasonably anticipated to be a human carcinogen [10]. An increase in the frequency of mutations by 1,2-dichlorobenzene was reported in an auxotrophic strain of Aspergillus nidulans. Chromosome studies in workers occupationally exposed for 4 days (8 hours/day) to 1,2-dichlorobenzene vapors (the concentration was thought to have exceeded 100 ppm), showed a statistically significant increase in the incidence of chromosomal alterations (in chromosomes isolated from peripheral blood cells) when compared to chromosomes isolated from the blood cells of a control population. The number of single and double chromosome breaks was also increased [11]. Toxicity of naphthalene and its metabolites, 1-naphthol and 2-naphthol was studied by DNA fragmentation assay. It is reported that naphthols may cause DNA damage on human lymphocytes [12]. Because of the known carcinogenicity and genotoxicity, the presence of residual 1-Naphthol and 1,2-Dichlorobenzene in Sertraline hydrochloride drug substance must be checked or controlled as per European Medicines Agency (EMA),

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International Council for Harmonization [13, 14] and Food and Drug Administration (FDA) guidelines [15]. The limit of genotoxic impurities is calculated on the basis of the threshold of toxicological concern” (TTC) concept. The daily recommended maximum dose of Sertraline hydrochloride is 0.2 g per day [16, 17]. 1-Naphthol and 1,2-Dichlorobenzene need to be controlled at a limited 7.5 μg/g in drug substance. To achieve quantification of such a trace level, it is necessary to develop sensitive, accurate and robust analytical method.

Sertraline hydrochloride is an official drug substance in the United State Pharmacopoeia (USP) and European Pharmacopoeia (EP). Both compounds 1-Naphthol and 1,2-Dichlorobenzene are not controlled in the official monograph. During literature survey, several analytical methods were reported for estimation of either 1-Naphthol or 1,2-Dichlorobenzene. But simultaneous detection and quantification of both compounds in pharmaceutical products are not reported. Quantification of Naphthalene and its metabolites, 1-naphthol and 2-naphthol in urine is reported with largely automated sample processing. It is three-dimensional high-performance liquid chromatography with triple column switching mode by fluorescence detection [18-20]. 1,2-dichlorobenzene is estimated in blood by extraction with n-heptane and is followed by gas chromatographic separation either on packed or capillary columns and quantitation with an electron capture detector (ECD) [21]. Trace level determination of chlorobenzenes in sediments is performed by GCMS [22]. Volatile organic compounds in drinking water are reported in US EPA method by GCMS [23]. HPLC determination of chlorobenzenes, benzene-sulphonyl chlorides and benzene-sulphonic acids in industrial wastewater is also reported [24]. Efficient HPLC separation of alkyl benzenes, naphthalene and aniline compounds is documented using specialized MIL-53(Fe) packed column [25]. Here, only standards are tested but sample or matrix interference is not studied. Literature suggests that to achieve such a low level of limit of quantification, hyphenated techniques with sophisticated instruments like GC-MS or LC with fluorescence detector is required. It is hardly possible for quality control laboratory of the pharmaceutical industry to perform testing routinely with GC-MS because of the cost and maintenance of a mass detector. In the present research, an analytical method is developed to quantify the 1-Naphthol and 1,2-Dichlorobenzene simultaneously at trace level using HPLC-UV detector. Both impurities are quantified and controlled in the key starting material of the sertraline hydrochloride that is in Schiff base. The proposed analytical method is validated as per International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines ICH Q2-R1 [26].

2. MATERIALS AND METHODS

2.1. Instrumentation

Shimadzu high-performance liquid chromatography (HPLC) system LC-2010 CHT with UV detector with LC solutions software or its equivalent.

2.2. Chemical and Reagents

HPLC column was used from Nacalai Tesque Inc. with Cosmosil C18, length 100 mm, internal diameter 4.6mm, particle size 3μm. 1-Naphthol standard of purity 99% was procured from SD Fine, 1,2-Dichlorobenzene standard of purity 99% from Spectrochem, methanol HPLC grade of Merck, Sodium perchlorate monohydrate AR grade from Loba Chemie and purified HPLC grade water was used in the experiment.

2.3. Chromatographic Parameters

HPLC method was performed using Cosmosil C18 HPLC column. Separation and peak symmetry were achieved with the mixture of mobile phase-A: buffer and mobile phase-B: a mixture of buffer and methanol (10:90 v/v) in gradient elution with timed programme Tmin/A:B: T0/30:70; T10/30:70; T17/00:100; T22/00:100; T35/30:70 and T 35/30:70 with flow rate 1.0 mL/min. The column temperature was maintained at 45°C and autosampler temperature was 15°C. Ultraviolet detection was performed at 210 nm. Injection volume was 50 μL, injector rinsing solution was methanol and run time was 35.0 minutes.

2.4. Preparation of Buffer

A solution of 0.01M Sodium perchlorate in 1000 mL of water was prepared. The solution was filtered through 0.45μ filter paper and sonicated to degas.

2.5. Solution Preparation

2.5.1. Diluent Preparation

A mixture of equal quantity of water and methanol was prepared.

2.5.2. Standard Stock Solution A

50 mg of 1,2-Dichlorobenzene standard was accurately weighed and transferred into 100 mL volumetric flask. The
achieve the trace level of 7.5 ppm. On the other hand, to vaporized, getting a maximum response is difficult to cannot be achieved and if the analyte is not completely overhead space GC because in headspace, more than 100 high boiler in nature, liquid injection technique was preferred over reverse phase RP-HPLC as a starting point. Due to a simultaneous detection of both impurities, GC was preferred chemical properties of the compounds and to achieve organic solvents and insoluble in water. On the basis of Naphthol is 288/g1.

3.1. Analytical Method Development

3. RESULTS AND DISCUSSION

3.1. Analytical Method Development

During literature survey, it was found that most of the methods for determination of 1,2-Dichlorobenzene (ODCB) are by GCMS. The boiling point of ODCB is 180°C and 1-Naphthol is 288°C. Both the compounds are soluble in organic solvents and insoluble in water. On the basis of chemical properties of the compounds and to achieve simultaneous detection of both impurities, GC was preferred over reverse phase RP-HPLC as a starting point. Due to a high boiler in nature, liquid injection technique was preferred overhead space GC because in headspace, more than 100°C cannot be achieved and if the analyte is not completely vaporized, getting a maximum response is difficult to achieve the trace level of 7.5 ppm. On the other hand, to achieve less limit at parts per million (ppm) sample, concentration is required more in grams. Secondly, key raw material of sertraline hydrochloride that is Schiff base, has a very high boiling point for which GC is not a suitable technique. Considering these properties of analyte and product, we decided to use heptanes as diluent because both impurities are freely soluble in heptanes but Schiff base is practically insoluble. Using different stationary phases, both analytes were resolved base to base using DB-624 column of length 30 m, internal diameter 0.53 mm, film thickness 3µm and peaks being symmetrical. ODCB was eluted at a retention time (RT) of 4.8 minutes and peak area at low level 7.5 ppm which was found sufficient to get reproducibility. 1-Naphthol peak was eluted at RT 12 minutes but peak response was very less due to a high boiling point in nature. So, it was observed that simultaneous estimation of both compounds is not possible with GC-FID (flame ionization detector). It can be possible with sophisticated instruments with hyphenated techniques like GC-MS (mass detector). To develop simple, sensitive, accurate and cost-effective method, we decided to change the technique to liquid chromatography (LC) because both impurity structures have chromophores.

Normal phase liquid chromatography development was started on the basis of solubility of the analyte and matrix. As explained earlier, both analytes are soluble in n-heptane and Schiff base sample is insoluble. Because of insolubility of the matrix, column load is reduced so, first the trial was taken on silica column of a dimension 250 mm x 4.6 mm, 5µm. Both impurities were injected with various compositions of isopropyl alcohol and n-hexane mobile phases but both analytes were not retained on the column. In a non-polar mobile phase, 100% n-hexane, also both peaks were eluted at 3.0 minutes so obviously, no resolution between analytes was obtained. In place of silica column, mid-polar cyano column was tried but still, peaks were not retained. In reverse phase, HPLC with a UV detector on C8 and C18 column, both analytes were separated. On Inertsil ODS 3V column of a dimension, 250 mm x 4.6 mm, 5µm with mobile phase water and methanol in a gradient mode of elution, 1-Naphthol, and ODCB peaks were eluted at 6.2 and 11.9 minutes, respectively but ODCB peak was asymmetrical. To improve the peak shape of ODCB, few trials were taken by replacing water with acidic buffer 0.1% phosphoric acid. Further, methanol was replaced with acetonitrile; the symmetrical peak of the ODCB was achieved but the Schiff-base peak was split into two peaks and one of them was eluted at 2.1 minutes and another at 16.2 minutes which interfered with both analytes. In the next trial with the same Inertsil ODS 3C column, acetonitrile was replaced by methanol and single peak of the Schiff-base was obtained but again the ODCB peak was asymmetrical. By keeping the same mobile phase, the column was changed to Cosmosil C18 of a dimension 250 mm x 4.6 mm, 5µm and achieved the symmetry of the ODCB peak. The response of both peaks at low level was also sufficient with the relative standard deviation (RSD) of precision less than 2.0. Due to the high concentration of the sample, matrix interference was observed at the RT of both analytes. For further separation of matrix interference, 0.01M sodium perchlorate was added to aqueous part of the mobile phase. Both impurities were
Quantification of Genotoxic Impurities in Key Starting Material
Retained on the column, and interference of the matrix peaks was also resolved but the runtime of the method was increased to 60 minutes which is time-consuming. To reduce the runtime, a column was changed to a shorter length, and particle size was also reduced to get better separation and gradient elution was optimized to elute out matrix peaks early. The desired goal was achieved with stationary phase Cosmosil C18, length 100 mm, internal diameter 4.6 mm, particle size 3 μm where run time was reduced to 35.0 minutes without baseline and matrix peak interference at the RT of both analytes. Further, during method validation, the recovery of ODCB was observed on the higher side about 115% to 125% at LOQ level. The root cause was found that one very small unknown impurity of the sample was eluted in tailing of the ODCB peak. So after few trials, the column temperature was optimized to 45°C to resolve the impurity.

3.2. Analytical Method Validation

3.2.1. Specificity
Specificity is the ability to describe an adequate separation of a critical analyte from interferences of other materials likely to be present. Specificity of the method was proved by comparing blank, 1-Naphthol, 1,2-Dichlorobenzene, Schiff base, tetralone, acetone, benzene, toluene and mono-chlorobenzene separate injections. No interfering peak was observed at the retention time of 1-Naphthol and 1,2-Dichlorobenzene. 1-Naphthol and 1,2-Dichlorobenzene were well resolved from all other specified impurity peaks which are used in the manufacturing process of Schiff base. Refer to Figs. (2-4) of blank, 1-Naphthol and 1,2-Dichlorobenzene standard solution and spike solution chromatograms.

3.2.2. Solution Stability
Data to support the sample solution stability under normal laboratory conditions for the duration of the test procedure should be generated. Solution stability till eight hours of 1-Naphthol and 1,2-Dichlorobenzene had been checked by injecting the standard solution. 1-Naphthol and 1,2-Dichlorobenzene standard solution were prepared freshly before injection and immediately injected and the same solution was injected after every one-hour interval. The peak area of 1-Naphthol and 1,2-Dichlorobenzene of the freshly prepared standard solution was observed as 133385 and 36307 and after seven hours, it was 133419 and 31279, re-

![Fig. (2). Blank chromatogram.](image-url)

![Fig. (3). 1-Naphthol and 1,2-Dichlorobenzene standard solution chromatogram. Retention time of 1-Naphthol is 3.3 minutes and 1,2-Dichlorobenzene is 8.2 minutes.](image-url)

![Fig. (4). Chromatogram of Schiff base test solution is spiked with 1-Naphthol and 1,2-Dichlorobenzene standards at 100% level.](image-url)
spectively. No significant change in area was observed till seven hours. After eight hours, 1-Naphthol area was found stable but ODCB area was reduced to 26178.

3.2.3. Limit of Detection (LOD) and Quantification (LOQ)

Detection limit is the lowest concentration of the analyte in a sample that can be detected but not necessarily quantitated. The obtained LOD values of 1-Naphthol and 1,2-Dichlorobenzene are discussed.

LOD = 3.3 × σ / S

Quantitation limit is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

LOQ = 10.0 × σ / S

Where σ = the standard deviation of the response and S = slope of the calibration curve.

LOD and LOQ concentration are reported in parts per million (ppm) with respect to Schiff base test concentration. LOD of 1-Naphthol and 1,2-Dichlorobenzene is 0.075 ppm and 0.5 ppm and LOQ is 0.25 ppm and 1.5 ppm respectively. Precision of LOQ is checked by injecting six replicate injections of a mixture of both analytes at their LOQ level. Relative standard deviation (RSD) of the peak area of 1-Naphthol and 1,2-Dichlorobenzene at LOQ level is observed at 1.2% and 2.5% respectively which proves consistency and reproducibility of the method at the trace level.

3.2.4. Linearity

The linearity of the procedure is intended to give assurance to the analyst that the procedure being validated is capable of determining an accurate and precise quantity for the analyte over the range of expected measurement to be made. The linearity of analytes was checked over the range of LOQ level, 50%, 80%, 100%, 120% and 150% of the specified limit. The regression coefficients of 1-Naphthol and 1,2-Dichlorobenzene were 0.999 and 0.998, respectively. The linearity of 1-Naphthol and 1,2-Dichlorobenzene is plotted in Figs. (5 and 6) respectively. The horizontal x-axis or abscissa is typically chosen to represent an independent variable which is a concentration of the analyte. The vertical y-axis or ordinate is chosen to represent the dependent variable i.e. peak area, which changes as the independent variable is manipulated. The regression coefficient (r²) more than 0.99 is considered as evidence of an acceptable fit of the data to the regression line.

3.2.5. Accuracy

Accuracy is the measure of how close the experimental value is to the true value. This test evaluates the specificity of the method in the presence of the other known and unknown impurities under the chromatographic conditions used.

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**Fig. (5).** Linearity plot of 1-Naphthol.

**Fig. (6).** Linearity plot of 1,2-Dichlorobenzene.
for the analysis of the drug substance. The procedures can highlight recovery problems that could be encountered during the sample preparation and the chromatographic procedures. Analytical method may be considered validated in terms of accuracy if the mean value is within ±20% of the actual value. During recovery study, Schiff base batch was analyzed and then 1-Naphthol and 1,2-Dichlorobenzene was spiked in the Schiff base at LOQ level of 50%, 100% and 150% with respect to the limit of 1-Naphthol and 1,2-Dichlorobenzene. The recovery of 1-Naphthol was found at 89.7%, 100.4%, 90.7% and 100.8%, respectively and 1,2-Dichlorobenzene was 110.3%, 99.3%, 95.5% and 105.0%, respectively. It proves that the method is capable of quantifying both the analyte accurately and results will be reliable even at trace level.

3.2.6. Ruggedness Study

Ruggedness is nothing but evaluation of intermediate precision of the method. The attribute evaluates the reliability of the method in a different environment other than that used during the development of the method. The objective is to ensure that the method provides the same results when similar samples are analyzed once the method development phase is over. The method can be tested on multiple days, analysts, instruments, etc. The ruggedness of the method was evaluated by estimating % RSD of 1-Naphthol and 1,2-Dichlorobenzene standard solution tested by two different analysts on different days. % RSD of the area of 1-Naphthol and 1,2-Dichlorobenzene peak in standard solutions of both analysts should not be more than 10%. Six replicates of diluted standard solution were injected by each analyst. Relative standard deviation of the area of 1-Naphthol was found at 0.06% and 0.18% and 1,2-Dichlorobenzene was found 0.64% and 1.97% on two different days. This shows that there is no variation in day to day analysis. Ruggedness was checked using spiking solutions by two different analysts with six different preparations. 1-Naphthol and ODCB were spiked in the Schiff base test solution at 100% level. Finally, RSD of the total twelve preparations was calculated and found to be 1.4% of 1-Naphthol and 4.5% of ODCB. Further, the method was successfully applied to three Schiff base validation batches with 1-Naphthol and 1,2-Dichlorobenzene concentration levels were found far below the LOQ. Method validation summary is given in Table 1.

### CONCLUSION

The results of all validation parameters like system suitability, precision, specificity, accuracy, linearity of detector response, ruggedness and robustness were acceptable according to pharmacopeias and official guidelines. It indicates that the method is stable and suitable for the quantifica-

### Table 1. Analytical method validation summary.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Acceptance Criteria</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Selectivity</td>
<td>1-Naphthol and 1,2-Dichlorobenzene peaks should be well separated from all known and unknown peaks of Schiff base.</td>
<td>Complies. Method is selective.</td>
</tr>
<tr>
<td>2</td>
<td>Solution stability</td>
<td>Report Result</td>
<td>Solutions are stable up to 7 h</td>
</tr>
<tr>
<td>3</td>
<td>Limit of detection</td>
<td>Report Result</td>
<td>1-Naphthol = 0.075 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-Dichlorobenzene = 0.5 ppm wrt test concentration</td>
</tr>
<tr>
<td>4</td>
<td>Limit of quantification</td>
<td>Report Result</td>
<td>1-Naphthol = 0.25 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-Dichlorobenzene = 1.5 ppm wrt test concentration</td>
</tr>
<tr>
<td>5</td>
<td>LOQ precision</td>
<td>%RSD for six replicates of LOQ level standard solutions is NMT:10.0%</td>
<td>RSD of 1-Naphthol = 1.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-Dichlorobenzene = 2.5%</td>
</tr>
<tr>
<td>6</td>
<td>Linearity</td>
<td>Correlation: NLT 0.99</td>
<td>1-Naphthol = 0.999</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-Dichlorobenzene = 0.998</td>
</tr>
<tr>
<td>7</td>
<td>Accuracy</td>
<td>Recovery should be between 80% to 120%</td>
<td>complies</td>
</tr>
<tr>
<td>8</td>
<td>Method precision</td>
<td>%RSD for results of six spiked preparations is NMT:10%</td>
<td>RSD of 1-Naphthol = 0.06%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-Dichlorobenzene = 0.64%</td>
</tr>
<tr>
<td>9</td>
<td>Intermediate precision</td>
<td>%RSD for results of twelve spiked preparations (Method precision and Intermediate precision) is NMT:10%</td>
<td>RSD of 1-Naphthol = 1.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-Dichlorobenzene = 4.5%</td>
</tr>
<tr>
<td>10</td>
<td>Robustness-Column temperature</td>
<td>%RSD for Results of spike preparations in actual condition and changed condition is NMT:10%</td>
<td>RSD of 1-Naphthol = 1.6%</td>
</tr>
<tr>
<td></td>
<td>(43°C and 47°C)</td>
<td></td>
<td>1,2-Dichlorobenzene = 5.7%</td>
</tr>
</tbody>
</table>

Abbreviations: wrt=with respect to, NMT=not more than, NLT=not less than, h=hour, ppm=parts per million, RSD=relative standard deviation and LOQ=limit of quantification.
tion of 1-Naphthol and 1,2-Dichlorobenzene in the key raw material of Sertraline hydrochloride. Hence, the validated RP-HPLC analytical method with UV detector can be used for routine analysis of quantification of 1-Naphthol and 1,2-Dichlorobenzene in quality control laboratories in the pharmaceutical industry.

ETHICS APPROVAL AND CONSENT TO PARTICIPE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
Not applicable.

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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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