https://doi.org/ 10.33472/AFJBS.6.5.2024. 5842-5866

African Journal of Biological Sciences

Development and Validation of Gas Chromatography Method for the Determination of Potential Genotoxic Impurities in Sertraline hydrochloride

Amit B Bhosale[1*] Ravi Yadav[2] and Seema Kothari[1]

[1]Department of Chemistry, PAHER University, Udaipur, India
[2]Associste Director, R&D Center, Cipla, Mumbai, India

***Corresponding Author**
Amit B Bhosale
Research Scholar, Dept. of Chemistry,
PAHER University, Udaipur, India
Mob: 9967264443,
Email: amitbbhosale@yahoo.com

Article History
Volume 6, Issue 5, 2024
Received: 09 May 2024
Accepted: 17 May 2024
doi: *10.33472/AFJBS.6.5.2024. 5842-5866*

Abstract
A highly sensitive analytical gas chromatography (GC) method was developed for the simultaneous quantification of trace-level impurities of Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-Trichlorobenzene, and 1-Napthylamine in the drug substance of Sertraline Hydrochloride. These genotoxic impurities have been demonstrated to induce genetic mutation and chromosomal rearrangement and have the potential to cause cancer. The analysis was accomplished on a DB-17 (50%-phenyl)-methylpolysiloxane fused silica) (30-meter x 0.53mm, 1.0µ) GC analytical column using Helium as a carrier gas, flame ionization detector (FID) as a detector with detector temperature of 260°C and injector port temperature of 250°C, injection volume 1.0 µL with split ratio mode and column oven temperature for proper separation with respectable resolution. The method was validated for analytical parameters such as system suitability, specificity, linearity and range, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, and solution stability. The LOD and LOQ for Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-Napthylamine were determined to be 1.9 ppm and 6.25 ppm. The method demonstrated specificity in accurately identifying the target impurities, and precision studies confirmed its reproducibility and reliability. The method presents a simple and reliable solution for ensuring the quality and safety of pharmaceutical products containing sertraline hydrochloride.

Keywords: Chlorobenzene, 1,4-Dichlorobenzene, 1-Napthylamine, Sertraline Hydrochloride, 1,2,4-Trichlorobenzene

Introduction

The bulk of pharmaceutical agents originate from their elemental components (total synthesis) or the targeted alteration of naturally existing substances (biotransformation). Both methodologies necessitate the utilization of diverse chemical reagents, each capable of exerting specific transformations upon the substrate molecule. This reliance on a multitude of reactive agents introduces the potential for unintended consequences. Traces of by-products generated during the synthetic or biotransformation processes can frequently contaminate the final product, the active pharmaceutical ingredient (API), or the formulated drug product [1]. The utilization of diverse chemical entities during pharmaceutical synthesis can result in the inadvertent retention of trace remnants within the final product, leading to their presence as unwanted impurities [2]. The chemical reactivity of these residual contaminants can elicit a spectrum of detrimental effects, including DNA damage through genotoxicity and tumorigenesis via carcinogenicity, thereby jeopardizing human health [2,3]. The inadvertent presence of minute, chemically active molecules within active pharmaceutical ingredients (APIs), termed genotoxic impurities (GTIs), has emerged as a major point of contention for diverse stakeholders within the pharmaceutical landscape. Pharmaceutical companies, regulatory agencies like the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), patient populations, and healthcare professionals all share a heightened awareness of the potential adverse effects associated with these insidious contaminants. This collective recognition reflects the increasing gravity of this issue and underscores the necessity for decisive action [1,4].

Sertraline hydrochloride is the hydrochloride salt of sertraline, a synthetic derivative of naphthalenamine with anti-serotoninergic and anti-depressant properties. Sertraline selectively inhibits the neuronal uptake of serotonin (5HT), raising 5HT levels in the central nervous system (CNS). Sertraline is in a class of antidepressants called selective serotonin reuptake inhibitors (SSRIs). It works by increasing the amounts of 5HT, a natural substance in the brain that helps maintain mental balance. Sertraline is used to treat depression, panic attacks, obsessive-compulsive disorder, post-traumatic stress disorder, social anxiety disorder (social phobia), and a severe form of premenstrual syndrome (premenstrual dysphoric disorder). The mechanism of action of sertraline is presumed to be linked to its inhibition of CNS neuronal uptake of 5HT. Studies at clinically relevant doses in man have demonstrated that sertraline blocks the uptake of 5HT into human platelets. The use of sertraline in patients with hepatic disease should be approached with caution. A lower or less frequent dose should be used in patients with hepatic impairment. Sertraline should not be used in cases of severe hepatic impairment as no

clinical data are available. The maximum daily dosage of sertraline is 200 mg. Sertraline is eliminated from the body by other metabolic pathways to form ketone and alcohol, which are largely excreted really as conjugates. The elimination half-life of sertraline ranges from 22-36 hours, and once-daily administration is therapeutically effective.

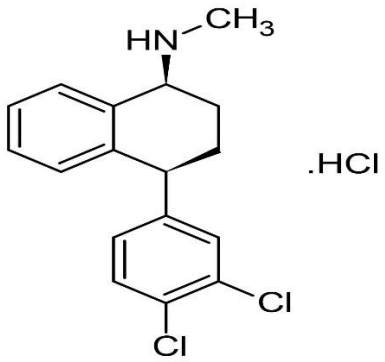


Figure 1: Chemical structure of Sertraline hydrochloride

Figure 1 depicts the chemical structure of sertraline hydrochloride. The sertraline hydrochloride drug is a white crystalline powder that is slightly soluble in water and isopropyl alcohol and sparingly soluble in ethanol**,** with molecular formula $C_{17}H_{17}N$ CL_2•HCL, molecular weight of 342.69, and pka values of 1.1 and 4.2. Sertraline hydrochloride is widely used and is effective in the action of treatment. Isolation and characterization of impurities are mandatory for acquiring and evaluating data that establishes biological safety, which reveals the need and scope for impurity profiling of drugs in pharmaceutical research. To isolate and quantify the impurities, various instrumental analytical techniques have been used routinely. Moreover, the recognition and regulatory contemplation of organic impurities is an extremely complex problem owing to numerous sources ranging from microbial contamination to degradation products of APIs apart from traces of intermediates. Although the International Council of Harmonization (ICH) has an out-lighted course of action about impurities, but still much more needs to be done. Hence, there is a strapping need to have unified specifications / standards about impurities. These GTIs are potential process and degradation impurities of the APIs. Because of its extreme toxicity, the regulatory authorities have made mandatory for the expectation of appropriate risk assessment and control strategy. A systematic review of the synthetic process and quality of the reagent is helpful for the control of such impurities. The objective of this work is to develop and validate simple, specific, precise, sensitive, linear, and accurate analytical methods for the determination of GTIs in APIs and further for drug products. Novel stability-indicating high-performance liquid chromatography (HPLC) and gas chromatography (GC)

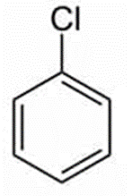
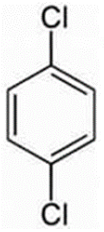
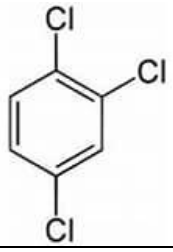
methods were developed to determine these GTIs in different classes of pharmaceutical compounds. The work also includes exploring the hyphenated technique of gas chromatography-mass spectroscopy (GCMS) for more sensitive and accurate detection and quantification of certain impurities. This study also demonstrated the suitability of developed methods in routine quality control applications by validating the developed methods as per USFDA and ICH guidelines requirements.

Materials and Methods:

Chemicals used:

Table 1 provides the sample information for all the GTIs used in this study, which were procured from Sigma-Aldrich (MO, USA). Deionized water was obtained using a Milli-Q water purification system (Millipore, MA, USA).

Table 1: Sample information on genotoxic impurities

S. No	**Chemical**	**Molecular formula**	**Molecular weight (g mol^{-1})**	**Structure**	**CAS No.**
1	Chlorobenzene	C6H5Cl	112.56		108-90-7
2	1,4-Dichlorobenzene	C6H4Cl2	147		106-46-7
3	1,2,4-Trichlorobenzene	$C_6H_3Cl_3$	181.44		120-82-1

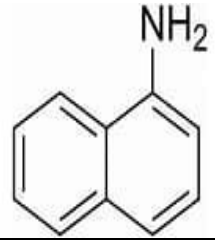
4	1-Napthylamine	$C_{10}H_9N$	141.19		134-32-7

Table 1 depicts the GTIs present in Sertraline hydrochloride. The Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-Napthylamine are process and carryover degrading GTIs of Sertraline hydrochloride found in In-process and final product of Sertraline hydrochloride drug substance (API). The method to determine this impurity is not official in any pharmacopeia, hence it was required to develop the In-house analytical method to estimate the content of Chlorobenzene, 1,4-Dichlorobenzene, 1,2,4-Trichlorobenzene and 1-Napthylamine in Sertraline hydrochloride by using GC instrument and also validate the same method as per the USFDA and ICH guidelines requirements guidelines.

Instrumentation

GC was conducted using an Agilent instrument (Model No. 6890N). A GC system capable of temperature programming was equipped with a capillary column, split/splitless Injector, a flame ionization detector, and an autosampler with suitable software. The suitable analytical column was DB-17 (50%-phenyl)-methylpolysiloxane fused silica) 30 m x 0.53 mm I.D. 1.0µ, employed for the analysis. Helium gas was selected as the carrier gas for chromatographic separation and operated at a constant flow rate of 6.5 mL min-1. The flame ionization detector (FID) served for detecting and quantifying the separated compounds, and helium gas was used as the makeup gas, flowing at a rate of 25 mL min-1. The temperature settings during the analysis were carefully controlled. The injector port was maintained at 250°C, the detector port was set at 260°C, and the column oven temperature was programmed as follows: Initially at 125°C and held at 2.0 min then increased at a rate of 15°C / min to 220°C and hold for 7.0 minute. Then increase with 15°C/min to 250°C and hold for 11.0

minutes. Each sample was injected with precision in a volume of 1 µL, and the analysis was performed in split mode with a split ratio of 1:10 to optimize the sensitivity and resolution.

Method Development

The main goal of this general approach is to detect small amounts of chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine in sertraline hydrochloride. The GC method is preferred owing to its excellent selectivity, as it analyses only the compounds that evaporate into the sample. Consequently, the GC method was studied further to determine the residual levels of chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzenet and 1-napthylamine in sertraline hydrochloride. For the development of this method, the capillary GC column DB-624, known for its suitability for analyzing various common ICH residual solvents in pharmaceutical products, was chosen. Several parameters, such as the sample diluent, temperature program, split ratio, oven temperature, and other GC conditions, were investigated and optimized using standard solutions. Table 2 represents the liquid conditions for AOC-20i analysis.

Table 2: Liquid conditions for AOC-20i analysis

Viscosity	:	0.2 [s]
Sample Wash	:	4
Pre-Solvent Wash	:	5
Solvent Wash	:	5
Inject Mode	:	Sample Air
Dispense Speed	:	Fast
Syringe Speed	:	Fast
Draw Speed	:	Fast
Vial Type	:	1_5mL
Rack Type	:	Short Rack
Pumps	:	5
Dwell Time	:	0.0 [s]
Syringe Height Up	:	0 [mm]
Syringe Height Down	:	0 [mm]
Syringe Height Inj	:	0 [mm]
Loads Per Inject	:	1
Air Gap	:	No
Solvent Select	:	A_only

Volume For Washing	:	80%
Wash Speed	:	Middle

Preparation of Standard and Sample Solutions

Solutions were prepared by accurately weighing chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine standards (0.1 g each). These were dissolved in 100 mL volumetric flasks containing 40 mL of diluent, diluted to the mark, and shaken at 1000 ppm. From each stock solution, 2.5 mL was further diluted to 100 mL with a 25 mL diluent (25 ppm). The dichloromethane and methanol at the ratio of 8:2 was taken as a diluent. The test solution was prepared by accurately weighing about 0.125 g of sample and transferring it to a 5 ml volumetric flask and adding 4.0 mL of diluent to it. It was then shaken and diluted to 5mL. The preparation of spiked sample solution was performed by weighing about 0.125 g of sample and transferring it to the 5 mL volumetric Flask and adding 4.0 mL of Standard solution. It was then diluted up to 5 mL. For the analysis, 1.0 µL of blank, standard solution (A) (6 replicates), standard solution (B), blank, identification solution of chlorobenzene, 1,4-dichlorobenzene, 1, 2, 4-trichlorobenzene and 1-napthylamine, sample solution, spiked sample solution, blank, and standard solution (A) (bracketing) were injected into the chromatographic system. Chromatograms were recorded, and peak responses were measured for GTIs in Sertraline-hydrochloride using the GC method.

System Suitability Test:

A system suitability test was performed to ensure the reliability and accuracy of the GC method, which aimed to assess the reliability and consistency of the chromatographic system. Working standard solutions (Working Standard solution-1 to Working Standard solution-6) were used for the test, with six replicate injections for each solution. The injection volume was 1.0 µL. The test evaluated two critical parameters: Relative standard deviation (RSD) for the peak area response (limit of 15.0 %) and RSD for retention time (limit of 2.0 %). Details of the samples and conditions used in a system suitability test are given in Table 2.

Calculation: Content of impurity in ppm

$$= \frac{(A - B) \times W1 \times 2.5 \times 5 \times P \times 100}{(C - B) \times 100 \times 100 \times W2 \times 100} \times \text{correction factor}$$

Where,

A = Peak area response of Respective Impurity in the test solution

B = Mean peak area response of Respective Impurity from Blank.

C = Mean peak area response of respective impurity in standard solution

P= Purity of Impurity used for the analysis.

W1 = Weight of impurity standard taken in g

W2 = Weight of sample in g

Correction factor for:

Chlorobenzene: 0.83

1,4- Dichlorobenzene: 0.84

1,2,4 TriChlorobenzene: 0.77

1-napthylamine: 0.88

Limit:

Chlorobenzene: Not more than 0.10%

1,4- Dichlorobenzene: Not more than 0.10%.

1,2,4 TriChlorobenzene: Not more than 0.10%.

1-napthylamine: Not more than 0.10%.

Table 3: Sequence table

Sample Name	No of Injections	Injection volume (µL)	Type of Testing
Blank	1	1.0	Blank
Blank	1	1.0	Blank
Blank	1	1.0	Blank
Working Standard solution-1	1	1.0	System suitability
Working Standard solution-2	1	1.0	System suitability
Working Standard solution-3	1	1.0	System suitability
Working Standard solution-4	1	1.0	System suitability
Working Standard solution-5	1	1.0	System suitability

Working Standard solution-6	1	1.0	System suitability
Blank	1	1.0	Blank
Test solution (As such)	1	1.0	Sample
Test solution (Spiked 100% Level)	1	1.0	Sample
Working Standard solution-Bkt.	1	1.0	System suitability

RESULTS

Specificity

The specificity of the method was confirmed by validating its ability to distinguish potential interference from blank, standard, sample, and spiked sample solutions. No detectable peaks were observed in the chromatograms of these solutions. Specifically, the chromatograms of the 25 ppm Chlorobenzene, 25 ppm 1,4-dichlorobenzene, 25 ppm 1,2,4-trichlorobenzene, and 25 ppm 1-Napthylamine-spiked sample solutions exhibited well-resolved peaks, which were clearly separated from all other peaks both before and after them. Additionally, the retention time of these GTIs in the chromatogram of the 25-ppm chlorobenzene, 25 ppm 1,4-dichlorobenzene, 25 ppm 1,2,4-trichlorobenzene, and 25 ppm 1-napthylamine spiked sample solutions closely matched that of the corresponding 25 ppm chlorobenzene, 25 ppm 1,4-dichlorobenzene, 25 ppm 1,2,4-trichlorobenzene, and 25 ppm 1-napthylamine standard solutions, as depicted in Figure 2-9.

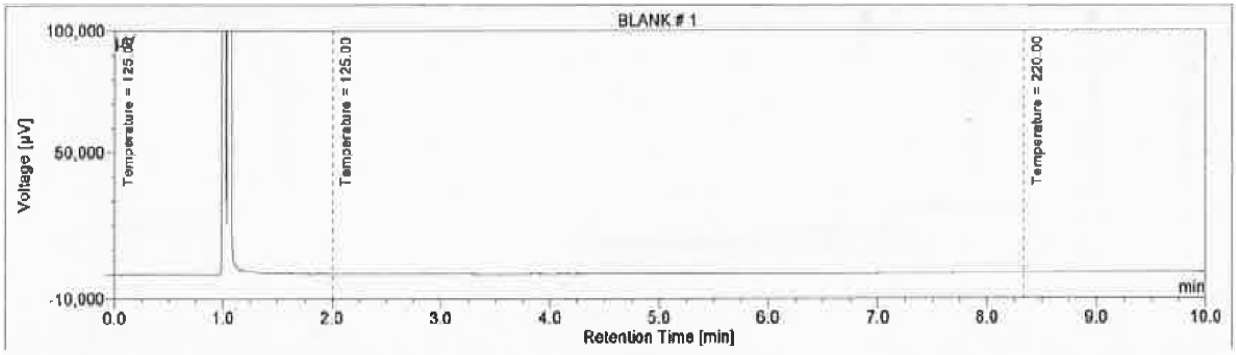


Fig.2: Blank chromatogram

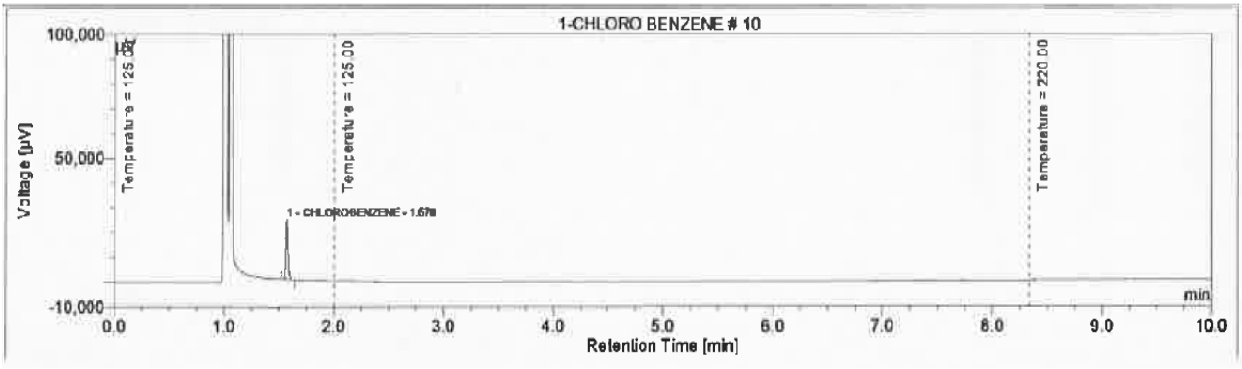


Fig.3: Identification solution of 1-chlorobenzene chromatogram

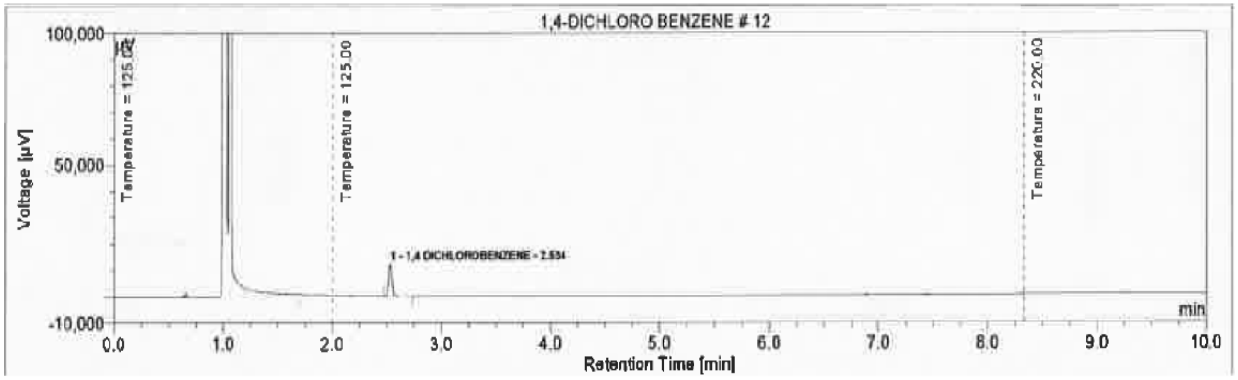


Fig.4: Identification solution of 1,4-dichlorobenzene chromatogram

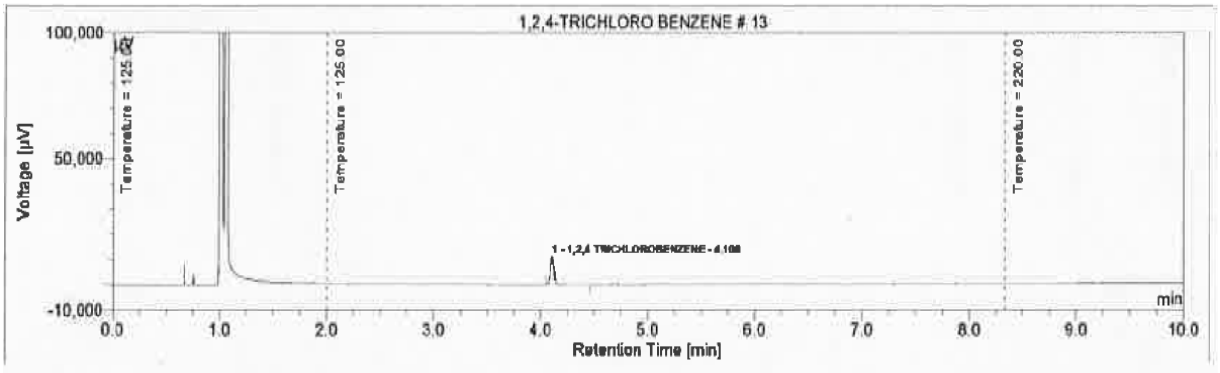


Fig.5: Identification solution of 1,2,4-trichlorobenzene chromatogram

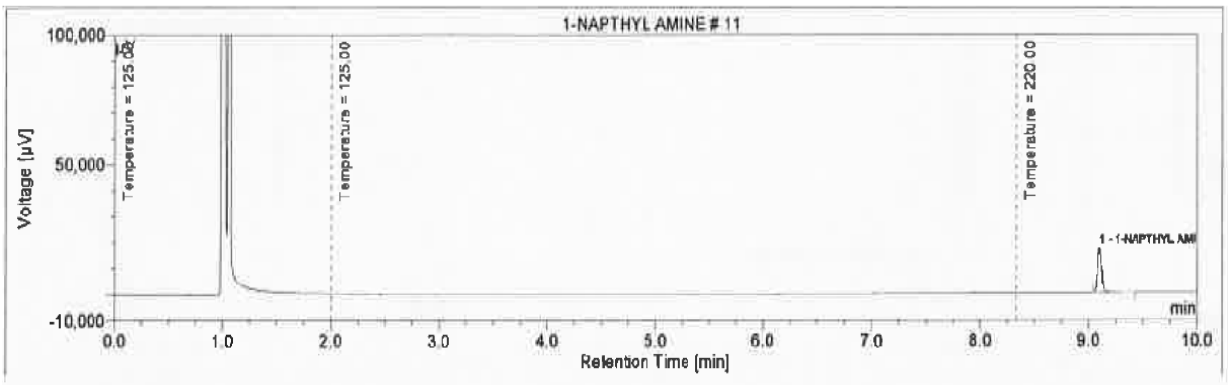


Fig.6: Identification solution of 1-napthylamine chromatogram

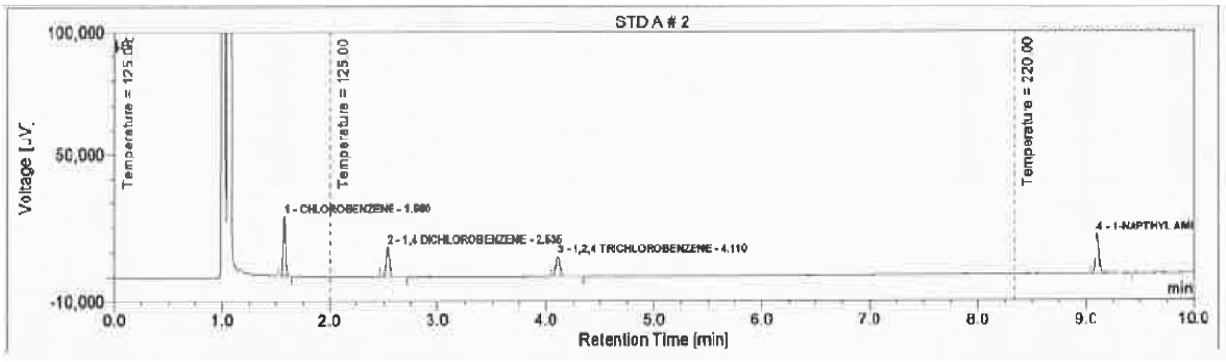


Fig.7: Mixture of standard chromatogram (Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1-napthylamine)

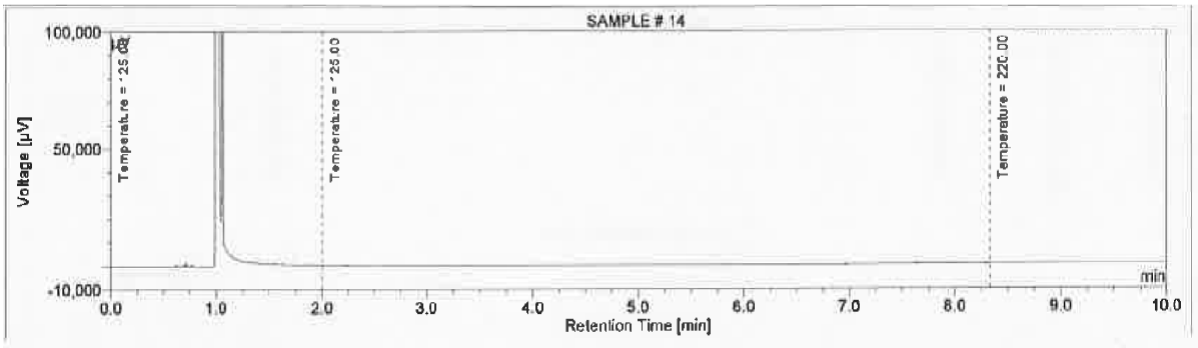


Fig.8: Sample of sertraline hydrochloride (As such) chromatogram

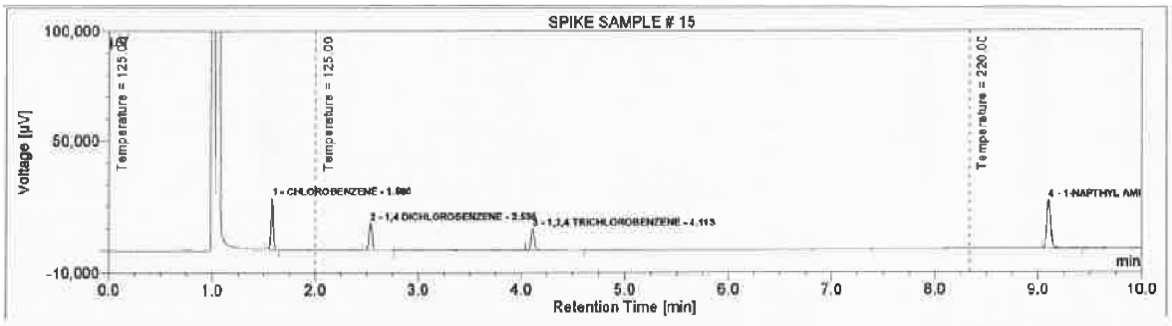


Fig.9: Sample of sertraline hydrochloride spiked with 100% chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine at limit level.

System Precision

The precision of the method was determined by system precision (six replicate injections of standard solution) and method precision (six different preparations of spike solution) studies. The peak areas of the analytes were measured for each standard injection. The % RSD was calculated based on the peak area data from these repeated standard injections to

evaluate the precision of the system. In both the studies % RSD of peak areas for all the solvents was less than 15.0%. The % of RSD is shown in Table 4.

Table 4: Percentage of Relative Standard Deviation

Sr. No	Levels	Chlorobenzene		1,4-Dichlorobenzene		1,2,4-Trichlorobenzene		1-Napthylamine	
		Retention time	Area	Retention time	Area	Retention time	Area	Retention time	Area
1	Standard	1.580	35108	2.535	24245	4.110	20220	9.105	44592
2	Standard	1.580	34675	2.535	23947	4.110	19927	9.103	44154
3	Standard	1.580	34355	2.535	23781	4.110	19899	9.103	43939
4	Standard	1.581	34975	2.535	24200	4.112	20305	9.106	44871
5	Standard	1.581	35425	2.535	24427	4.111	20357	9.105	45608
6	Standard	1.581	34701	2.536	24151	4.111	20250	9.105	44633
	Mean	1.581	34873	2.535	24125	4.111	20160	9.105	44633
	SD	0	376.15	0	228.88	0	196.95	0	586.52
	% RSD	0.03	1.08	0.02	0.95	0.02	0.98	0.01	1.31

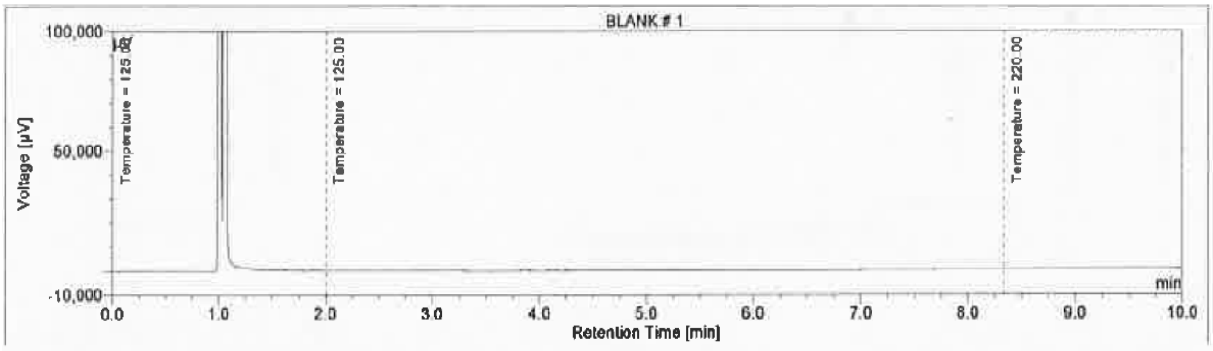


Fig.10 Blank chromatogram

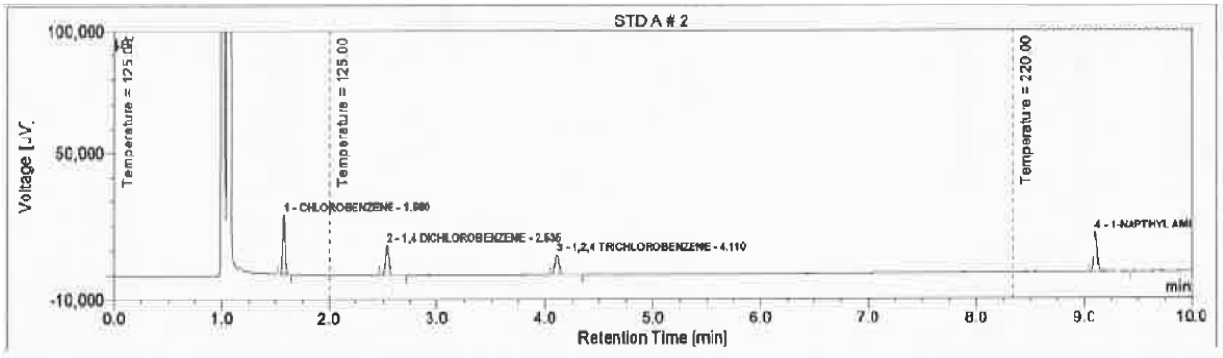


Fig.11 Mixture of Standard Chromatogram (Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1-napthylamine)

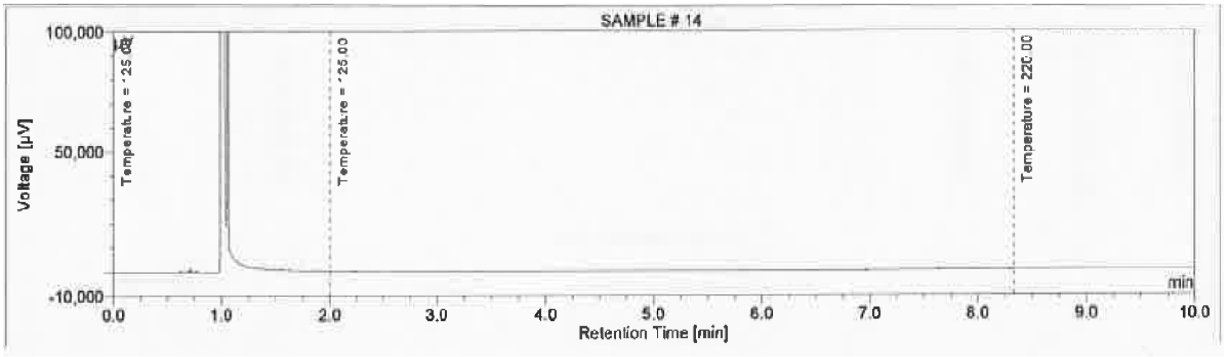


Fig.12 Sample (As such) chromatogram of sertraline hydrochloride

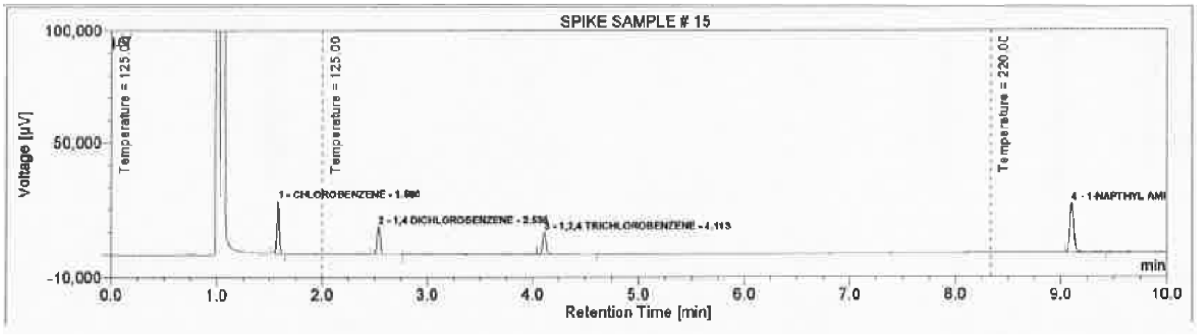


Fig.13 Sample of sertraline hydrochloride spiked with 100% chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine at limit level

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analysts in a sample which can be detected but not necessarily quantitated as an exact value. In this study, the LOD values were determined by examining the signal-to-noise ratio for each impurity. The analysis of standard solutions with varying concentrations of impurities was used to obtain the LOD values. The chromatogram in Figure 14 illustrates the LOD for each

impurity. Table 5 provides a summary of the limit of detection (signal-to-noise ratio) for the different impurities at their respective concentrations.

Table 5: Limit of detection (Signal-to-noise ratio)

Sr. No	Impurities	Concentration (ppm)	Signal to noise
1	Chlorobenzene	1.9	100
2	1,4-Dichlorobenzene	1.9	100
3	1,2,4-Trichlorobenzene	1.9	131
4	1-Napthylamine	1.9	23

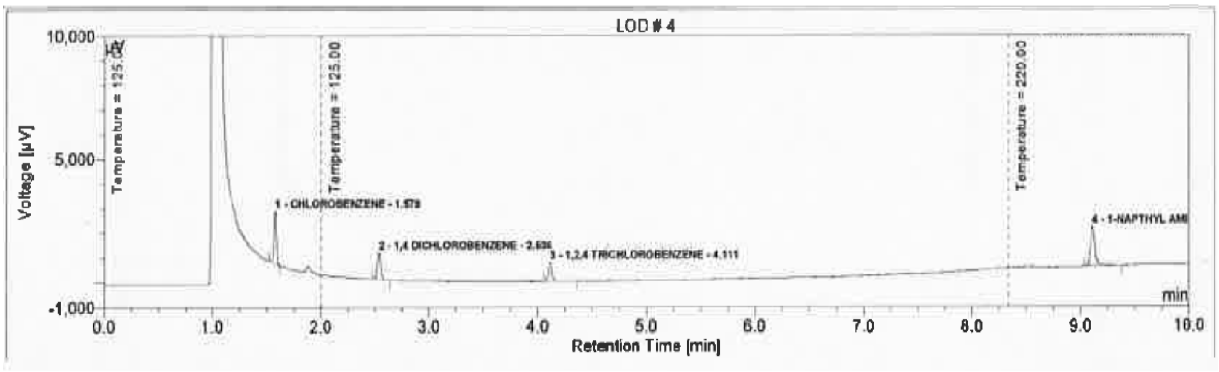


Fig.14 Limit of detection chromatogram

Limit of Quantification (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOQ was determined for each impurity. The LOQ values were calculated based on the signal-to-noise ratio obtained from the analysis of the standard solutions with varying concentrations of impurities. The results are presented in Table 6. The precision at the LOQ level was assessed by injecting three replicate injections of the LOQ standard solution for each impurity, including Chloromethane, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine. The data obtained for the retention time and peak area of each impurity at the LOQ is presented in Table 7.

The % RSD values represent the precision of the GC method at the LOQ for each impurity. The low % RSD values indicate good precision and repeatability of the method, ensuring the accurate and reliable quantification of trace impurities in the samples. These results demonstrate the robustness and suitability of the GC method for the determination of

impurities at low concentrations; thereby providing valuable insights for analytical applications. The chromatogram in Figure 15 illustrates the LOQ for each impurity.

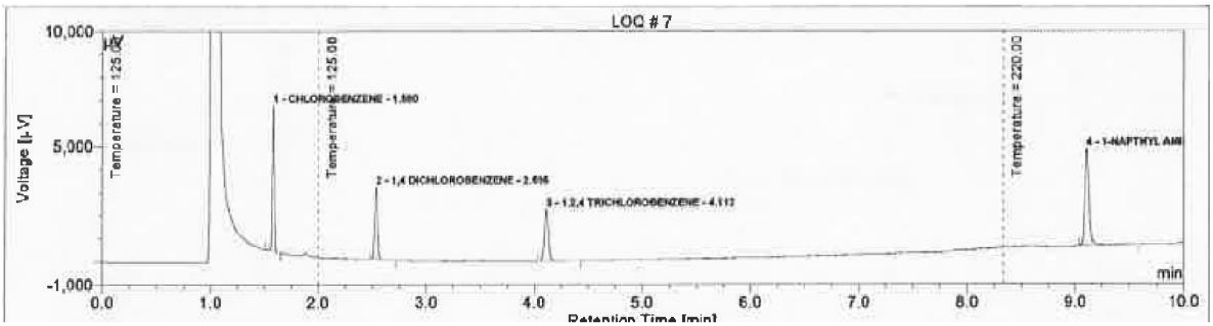


Fig.15 Limit of quantification chromatogram

Table 6: Limit of Quantitation (Signal to noise ratio)

Sr. No	Impurities	Concentration (ppm)	Signal to noise
1	Chloromethane	6.25	200
2	1,4-Dichlorobenzene	6.25	350
3	1,2,4-Trichlorobenzene	6.25	370
4	1-Napthylamine	6.25	60

Precision at LOQ is also performed by injection of three replicate injections of LOQ standard solution and data: Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine

Table 7: Limit of Quantitation (% RSD for chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1-napthylamine)

Sr. No	Levels	**Chlorobenzene**		**1,4-Dichlorobenzene**		**1,2,4-Trichlorobenzene**		**1-Napthylamine**	
		Retention time	Area	Retention time	Area	Retention time	Area	Retention time	Area
1	LOQ	1.580	8948	2.535	6239	4.11	5337	9.105	11375
2	LOQ	1.580	8721	2.535	6180	4.11	5152	9.103	11109
3	LOQ	1.580	8864	2.535	6039	4.11	5167	9.103	10815
	Mean	1.580	8844.3	2.535	6152.7	4.11	5218.7	9.104	11099.7
	SD	0.00	114.8	0.00	102.8	0.00	102.8	0.00	280.1
	% RSD	0.00	1.3	0.00	1.7	0.02	2.0	0.01	2.5

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 (amount) of analyte in the sample. The linearity of chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine was determined. Table 8 presents the linearity level preparations for each analyte, including the volume of the standard stock solution used with corresponding concentrations in ppm, and as a percentage concerning the sample. Table 9 displays the results of the linearity level correlation coefficient and % Y-intercept for each analyte. The correlation coefficient indicates the strength of the linear relationship between the analyte concentration and its corresponding peak area. High correlation coefficients close to 1 indicate a strong linear relationship between the concentration and the peak area for each analyte. The % Y-intercept values represent the extent of deviation from zero on the Y-axis, indicating a minimal bias in the calibration line. The linearity level I, II, III, and IV are represented by Figures 16-19.

Standard Stock Solution: Further transfer 2.5 mL of the standard stock of chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1-napthylamine to a 100 mL volumetric flask containing about 25 mL of diluent and dilute it up to the mark with the diluent.

Table 8: Linearity level preparation (Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1-napthylamine)

	Volume of Standard Solution to be taken in (mL)	**Volume of Standard Stock Solution to be taken in (mL)**	**Volume to be diluted with Diluent (mL)**	**Concentration of Linearity level solution (ppm)**	**Corrected concentration of Linearity level solution (ppm) w.r.t Sample**
LOQ 25% (LIN I)	12.5	-	50	6.25	250
50 % (LIN II)	-	1.25	100	12.5	500
100 % (LIN III)	-	2.50	100	25.0	1000
150 % (LIN IV)	-	3.75	100	37.5	1500

Table-9: Linearity level Correlation Coefficient and % Y intercept (Chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1-napthylamine)

Level	**Chlorobenzene**		**1,4-dichlorobenzene**		**1,2,4-trichlorobenzene**		**1-napthylamine**	
Level	**Concentration in (ppm)**	**Mean Area**	**Concentration in (ppm)**	**Mean Area**	**Concentration in (ppm)**	**Mean Area**	**Concentration in (ppm)**	**Mean Area**
Level-I	6.25	8844	6.25	6153	6.25	5219	6.25	11100
Level-II	12.5	17373	12.5	12027	12.5	10135	12.5	22536
Level-III	25.0	34403	25	23961	25	20156	25	45186
Level-IV	37.5	51940	37.5	36226	37.5	30347	37.5	67835
	Regression coefficient	0.999	Regression Coefficient	0.999	Correlation Coefficient	0.999	Correlation coefficient	0.999
	% Y intercept	-3.3	% Y intercept	-3.6	%Y Intercept	-3.1	% Y Intercept	-4.2

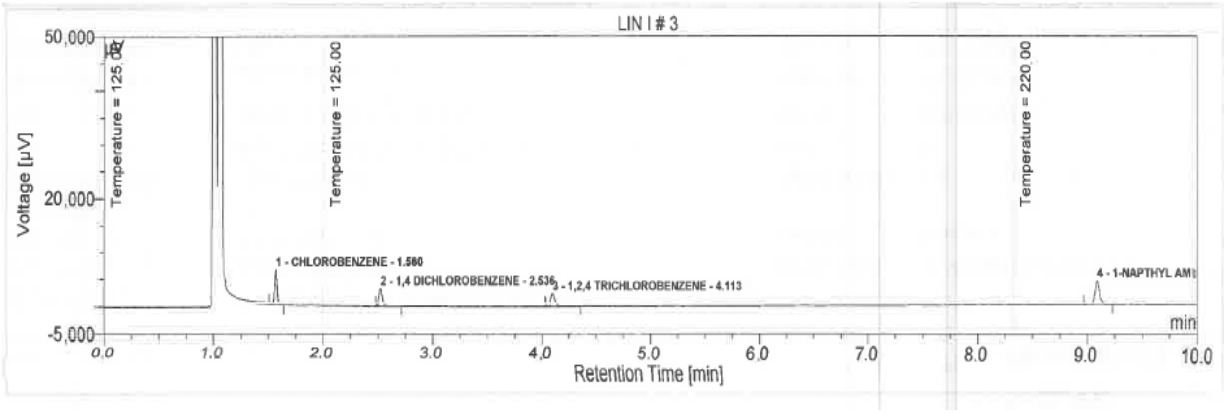


Fig.16 Linearity level-I (LOQ) chromatogram

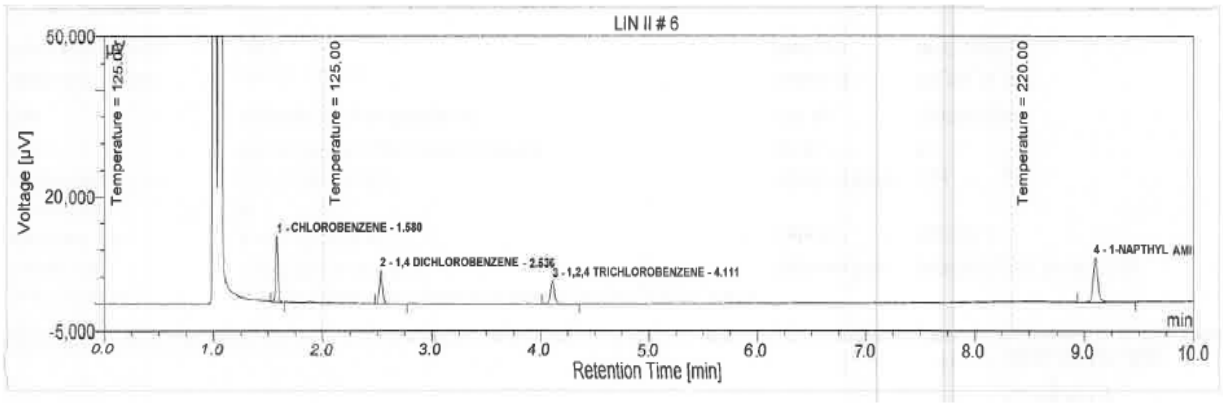


Fig.17 Linearity level-II (50 %) chromatogram

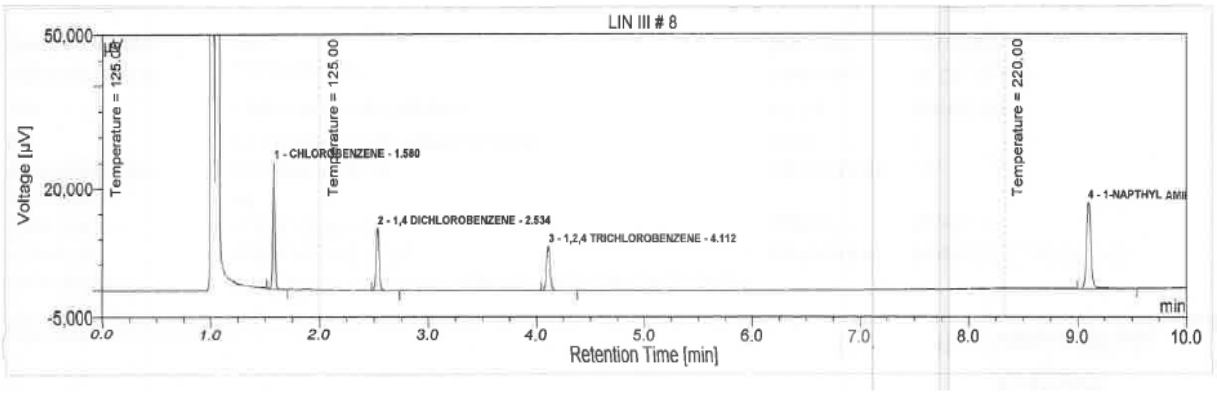


Fig.18 Linearity level-III (100 %) chromatogram

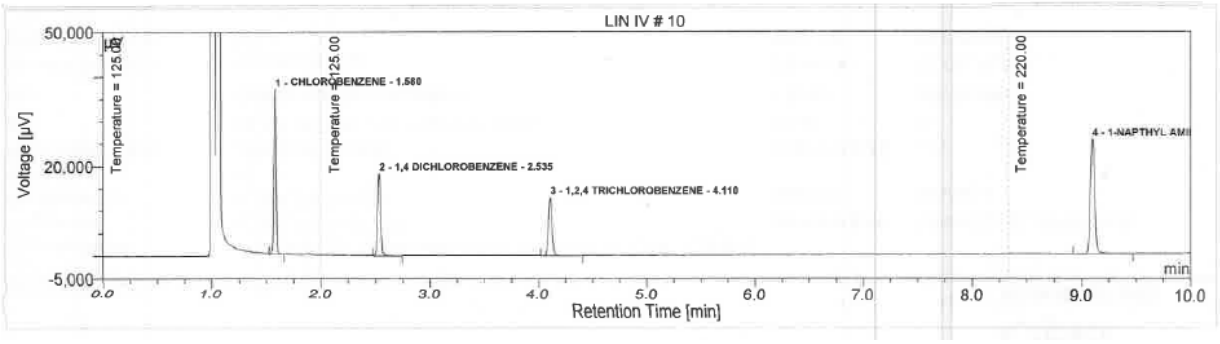


Fig.19 Linearity level-IV (150 %) chromatogram

ACCURACY:

The accuracy of the GC method was evaluated to determine the closeness of agreement between the measured values and the accepted reference values for chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine. Accuracy assessment was performed at various concentration levels, including the LOQ, (50 %), (100 %), and (150 %) limit level. Duplicate preparations were performed at each concentration level to ensure

reliable and robust results. The accuracy results demonstrated the closeness of the measured values to the reference values at each accuracy level. The calculated accuracy values for all analytes showed high agreement with the reference values, as the percentages were close to 100%, indicating excellent accuracy of the GC method. Tables 10, 11, 12, and 13 summarize the accuracy results for each analyte, presenting the mean area standard obtained for the samples along with the corresponding peak areas at different accuracy levels. Figures 20-23 represent the accuracy at LOQ, 50, 100, and 150% level chromatograms of sertraline hydrochloride.

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy performs at LOQ, Limit level (100%), and 150% level in duplicate preparation.

Accuracy table for Chlorobenzene, 1,4-Dichlorobenzene, 1,2,4-trichlorobenzene and 1-Napthylamine

Table 10: Accuracy of Chlorobenzene:

Sr. No	Sample Name	Area observed in Std	Area observed in accuracy sample	Amount added	Amount found	% Recover (Amount found/Amount Added*100)
1	ACC @ LOQ-1/1	35693	9211	0.030	0.026	86.0
2	ACC @ LOQ-1/2		9316	0.030	0.026	87.0
3	ACC @ 50% 1/1		17562	0.050	0.049	98.4
4	ACC @ 50% 1/2		17236	0.050	0.048	96.6
5	ACC @ 100% 1/1		36219	0.100	0.101	101.5
6	ACC @ 100% 1/2		36846	0.100	0.103	103.2
7	ACC @ 150% 1/1		51369	0.150	0.144	95.9
8	ACC @ 150% 1/2		51894	0.150	0.145	96.9

Table 11: Accuracy of 1,4-dichlorobenzene:

Sr. No	Sample Name	Area observed in Std	Area observed in accuracy sample	Amount added	Amount found	% Recover (Amount found/Amount Added*100)
1	ACC @ LOQ-1/1	24558	6412	0.030	0.026	87.0
2	ACC @ LOQ-1/2		6539	0.030	0.027	88.8
3	ACC @ 50% 1/1		12593	0.050	0.051	102.6
4	ACC @ 50% 1/2		13682	0.050	0.056	111.4
5	ACC @ 100% 1/1		26549	0.100	0.108	108.1
6	ACC @ 100% 1/2		26103	0.100	0.106	106.3
7	ACC @ 150% 1/1		37591	0.150	0.153	102.0
8	ACC @ 150% 1/2		37159	0.150	0.151	100.9

Table 12: Accuracy of 1,2,4-Trichlorobenzene:

Sr. No	Sample Name	Area observed in Std	Area observed in accuracy sample	Amount added	Amount found	% Recover (Amount found/Amount added*100)
1.	ACC @ LOQ-1/1	21135	5421	0.030	0.026	85.5
2.	ACC @ LOQ-1/2		5569	0.030	0.026	87.8
3.	ACC @ 50% 1/1		10692	0.050	0.051	101.2
4.	ACC @ 50% 1/2		9963	0.050	0.047	94.3
5.	ACC @ 100% 1/1		18369	0.100	0.087	86.9
6.	ACC @ 100% 1/2		18963	0.100	0.090	89.7
7.	ACC @ 150% 1/1		30016	0.150	0.142	94.7
8.	ACC @ 150% 1/2		30139	0.150	0.143	95.1

Table 13: Accuracy of 1-Napthylamine:

Sr. No	Sample Name	Area observed in Std	Area observed in accuracy sample	Amount added	Amount found	% Recover (Amount found/Amount added*100)
1	ACC @ LOQ-1/1	45442	11976	0.030	0.026	87.8
2	ACC @ LOQ-1/2		11238	0.030	0.025	82.4
3	ACC @ 50% 1/1		23569	0.050	0.052	103.7
4	ACC @ 50% 1/2		23459	0.050	0.052	103.2
5	ACC @ 100% 1/1		43216	0.100	0.095	95.1
6	ACC @ 100% 1/2		43166	0.100	0.095	95.0
7	ACC @ 150% 1/1		55329	0.150	0.122	81.2
8	ACC @ 150% 1/2		55169	0.150	0.121	80.9

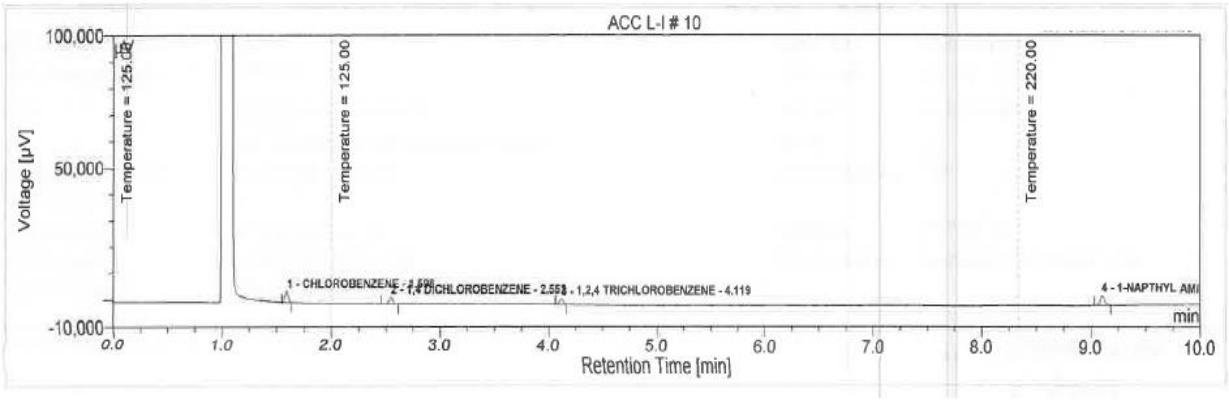


Fig.20 Accuracy @ LOQ level chromatogram of sertraline hydrochloride

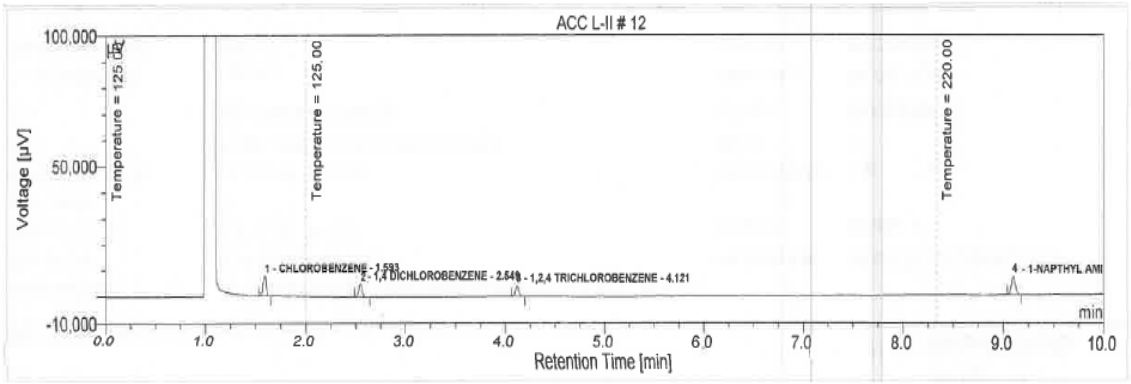


Fig.21 Accuracy @ 50% level chromatogram of sertraline hydrochloride

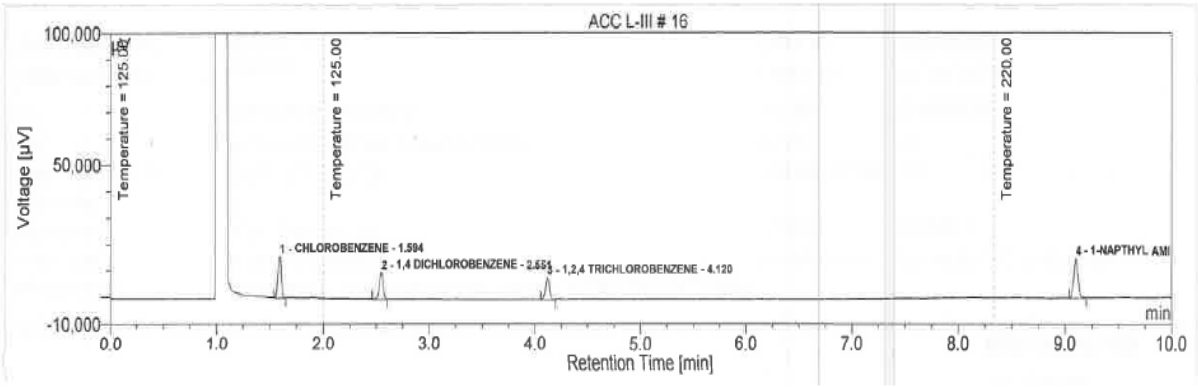


Fig.22 Accuracy @ 100% level chromatogram of sertraline hydrochloride

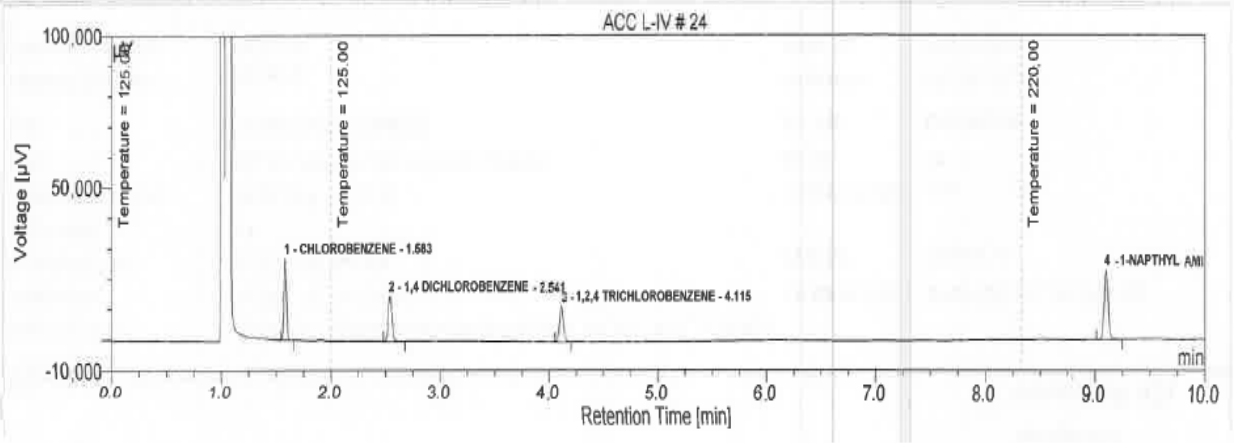


Fig.23 Accuracy @ 150% level chromatogram of sertraline hydrochloride

The obtained results are compared with the corresponding specified limits of ICH standard guidelines. The method validation was done by evaluating specificity, system suitability and method precision, LOD and LOQ, linearity, and accuracy of residual solvents as per the ICH guideline. All four GTIs are well separated from each other with good resolution and thus, the method was found to be specific. The precision of the method was determined by system precision (six replicate injections of standard solution) and method precision (six different preparations of spike solution) studies. In both studies % RSD of peak areas for all the solvents were less than 15.0%. The results proved that the system suitability was passed, and the method was precise.

The values of LOD and LOQ were much less than the lower limit of the concentration range. The linear relationship was evaluated across a range of LOQ to 150% of working level concentration. The graphs of theoretical concentration versus obtained concentration are linear

and the regression coefficients 'R' for residual solvents were more than 0.999 for all four GTIs. The accuracy of the method was determined by spiking of all four GTIs at four different level i.e. LOQ Level, 50% Level, 100% Level, and 150% Level, the Recovery of all four solvents for all four levels was found within the range of 80% to 120% of the limit level.

Overall, the results obtained in this study were compared to the specified limits of the ICH standard guidelines. The method was found to be specific, precise, sensitive, and accurate for the detection and quantification of GTIs, making it suitable for the determination of Chlorobenzene, 1,4-Dichlorobenzene, 1,2,4-Trichlorobenzene and 1-Napthylamine in Sertraline hydrochloride using GC. These findings validate the reliability and robustness of the developed method, which holds significant potential for quality control and safety assessments in pharmaceutical manufacturing processes.

Discussion

Impurities (GTIs) in sertraline hydrochloride were successfully validated and demonstrated reliable performance across various validation parameters. The results obtained in this study are consistent with and supported by previous research in analytical chemistry and pharmaceutical analysis. The specificity of the method was confirmed by the well-resolved peaks of all four GTIs, indicating its ability to accurately separate and quantify specific compounds in the presence of other matrix components. This finding aligns with previous studies where successful determination of specific impurities has been reported using GC [5,6].

The precision study revealed excellent reproducibility, both in terms of system precision and method precision, with % RSD values less than 15.0 % for all solvents. This precision was consistent with the findings of earlier works which also demonstrated the reliability and repeatability of GC methods in pharmaceutical analysis [7,8].

The LOD and LOQ values obtained in this study were significantly lower than the lower limits of the concentration range, indicating the high sensitivity of this method for detecting trace levels of GTIs. Similar results have been reported in previous studies on the quantification of impurities using GC [9,10]. The linearity study demonstrated a strong linear relationship between the theoretical and concentrations of GTIs were obtained with the regression coefficients (R) 0.999 for chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and 1-napthylamine compounds. These results are consistent with prior works [7,11,12] which reported the linearity of GC methods for the analysis of pharmaceutical compounds. The accuracy assessment involving spiking the GTIs at different levels showed

good recovery percentages, falling within the acceptable range of 80–120% of the limit level. This accuracy is consistent with the results reported in previous studies which also demonstrated accurate quantification of impurities using GC[11-13].

Overall, the results of this study, along with supporting references, confirm the suitability and reliability of the developed GC method for the determination of GTIs in sertraline hydrochloride. The specificity, precision, sensitivity, linearity, and accuracy of the technique make it a valuable analytical tool for quality control and safety assessment in the pharmaceutical industry. These findings contribute to the growing body of literature on the application of GC in pharmaceutical analysis and further validate its potential as a robust and efficient analytical technique.

Conclusion

The method was validated by critical principles stated in ICH guidelines, showing satisfactory data for all the method validation parameters tested. The developed method was validated by critical principles stated in ICH guidelines, showing satisfactory data for all the method validation parameters tested. The GC method was found to be simple, specific, precise, sensitive, linear, and accurate. Hence, the method can be successfully used in the pharmaceutical companies and research laboratories for the simultaneous determination of GTIs in Sertraline hydrochloride active pharmaceutical ingredients. The developed GC method for determining GTIs in Sertraline hydrochloride was successfully validated and proved effective.

Conflict of Interest

None

References:

1. Szekely G, Amores de Sousa MC, Gil M, Castelo Ferreira F, Heggie W. Genotoxic impurities in pharmaceutical manufacturing: sources, regulations, and mitigation. *Chemical reviews.* 2015;115(16):8182-8229.
2. Wu Y, Levons J, Narang AS, Raghavan K, Rao VM. Reactive impurities in excipients: profiling, identification and mitigation of drug-excipient incompatibility. *AAPS PharmSciTech.* 2011;12(4):1248-1263.
3. Müller L, Mauthe RJ, Riley CM, et al. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regulatory toxicology and pharmacology : RTP*. 2006;44(3):198-211.
4. Zhang K, Pellett JD, Narang AS, Wang YJ, Zhang YT. Reactive impurities in large and small molecule pharmaceutical excipients – A review. *TrAC Trends in Analytical Chemistry*. 2018;101:34-42.
5. Jadhav SA, Landge SB, Niphade NC, Bembalkar SR, Mathad VT. Development and Validation of Stability-Indicating GC-FID Method for the Quantitation of Memantine Hydrochloride and Its Nonchromophoric Impurities in Bulk and Pharmaceutical Dosages. *Chromatography Research International.* 2012;2012:806068.
6. Goswami N. A validated stability-indicating liquid chromatographic method for determination of process related impurities and degradation behavior of Irbesartan in solid oral dosage. *Journal of advanced pharmaceutical technology & research.* 2014;5(1):33-40.
7. Nacham O, Ho TD, Anderson JL, Webster GK. Use of ionic liquids as headspace gas chromatography diluents for the analysis of residual solvents in pharmaceuticals. *Journal of pharmaceutical and biomedical analysis.* 2017;145:879-886.
8. Sonawane SS, Chhajed SS, Attar SS, Kshirsagar SJ. An approach to select linear regression model in bioanalytical method validation. *Journal of Analytical Science and Technology*. 2019;10(1):1.
9. Wachełko O, Szpot P, Zawadzki M. A novel simple and precise method for the determination of azide impurity in sartans using headspace gas chromatography with two dissimilar capillary columns and two flame ionization detectors (HS-GC-FID/FID). *Journal of pharmaceutical and biomedical analysis.* 2021;192:113671.
10. Seyed Mojtaba M, Sussan G. Linearity of Calibration Curves for Analytical Methods: A Review of Criteria for Assessment of Method Reliability. In: Mark TS, ed. *Calibration and Validation of Analytical Methods.* Rijeka: IntechOpen; 2018:Ch. 6.
11. Han J, Yeung D, Wang F, Semin D. Determination of Residual Isobutylene Oxide—A Genotoxic Starting Material in a Drug Substance by Static Headspace Gas Chromatography. *Journal of chromatographic science.* 2008;46(7):637-642.
12. Venugopal N, Reddy AVB, Reddy KG, Madhavi V, Madhavi G. Method development and validation study for quantitative determination of 2-chloromethyl-3, 4-dimethoxy pyridine hydrochloride a genotoxic impurity in pantoprazole active pharmaceutical ingredient (API) by LC/MS/MS. *Journal of pharmaceutical and biomedical analysis.* 2012;70:592-597.
13. Reddy SR, Reddy KH, Kumar MN, Reddy PM, Reddy JVR, Sharma HK. A Validated GC-MS Method for the Determination of Genotoxic Impurities in Divalproex Sodium Drug Substance. *Journal of Chromatographic Science.* 2018;57(2):101-107.