RESEARCH ARTICLE

GC method development and validation of genotoxic impurity 1, 3 Dichloro propane, 3-chloro-1-bromopropane and 2Chloro pyridine content in Trazodone Hydrochloride API

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ABSTRACT

Trazodone is often used to treat major depression. A50 - 300 mg tablet formulation thatcontains1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridine as a genotoxic impurity. In the present study, the GC method is effectively developed and validated under the required regulations. This method is established for the quantification of 1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2 – Chloro pyridine in Trazodone hydrochloride API.The sample preparation method is easy and free of toxic solvents. The method demonstrated excellent linearity over a concentration range of 0.3 µg/ml to 2 µg/ml. The method was highly précised and robust. The proposed GC method holds promise for both qualitative and quantitative analyses of 1, 3 Dichloro propane, 3-chloro-1-bromopropane and 2-Chloro pyridine in Trazodone hydrochloride formulations.

Keywords: GC, Trazodone Hydrochloride, Genotoxic impurity

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INTRODUCTION

Trazodone hydrochloride is a 5-HT 2A receptor antagonistwhose IUPAC name is 2- {3- [4- (3-chlorophenyl)piperazin- 1- yl]propyl} [1,2,4]triazolo [4,3-a]pyridin- 3 (2 H)- one hydrochloride with chemical formula $C_{19}H_{23}Cl_2N_5O$. It is a physically solid white odorless crystalline powder with a drug content 98.0% - 102.0%.[1-12] The drug exhibits solubility in chloroform, is sparingly soluble in water, ethanol, and methanol, and remains insoluble in most common organic solvents.[13-18] Trazodone helps in enhancing mood, appetite, and energy levels while also alleviating anxiety and insomnia associated with depression. It functions by helping to restore the balance of serotonin, a natural chemical in the brain.[19-23] Trazodone induces sedation in patients having problems in sleeping and can lead to depression. It also improves apnea and hypopnea. Trazodone acts as a Serotonin reuptake inhibitor and serotonergic receptor, due to which it is used in treating anxiety, substance abuse, Alzheimer disease, substance abuse, behavioural problem in eating, and fibromyalgia.[2] It is also prescribed for the treatment of post-traumatic stress disorder (PTSD). Trazodone (dose: 50 mg to 200 mg) helps reduce the frequency of nightmares and improves sleep patterns in patients with PTSD.. According to various reported studies, Trazadone is not preferedas first line treatment because it intensifies the symptoms in panic case. However, several studies indicate that patients with panic symptoms are often better treated with SSRIs rather than trazodone for PTSD. Furthermore, studies have demonstrated that trazodone can reduce episodes of apnea and hypopnea in patients with obstructive sleep apnea (OSA) without exacerbating hypoxemic episodes.[3] It increases the respiratory threshold, thereby decreasing the risk of respiratory instability.[24,25] 1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridine are the impurities found in Trazodone hydrochloride which has genotoxic properties. At present, no chromatographical method is available for quantification as well as qualitative analysis for 1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridine present in Trazodonehydrochloride dosage form. In the present study, GC method developed and validated for 1, 3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridine content in Trazodone Hydrochloride.

MATERIALS & METHODS

Chemicals and equipment

All the chemicals were procured from Sigma-Aldrich, India, and utilized without further purification.GC analysis was performed on aGas chromatograph equipped with flame ionization detector. Shimadzu GC 2010plus, with AOC5000 autosampler. Separation wassuccessfully achieved on an DB-wax (30 m × 0.53 mm id,0.5µm) make: GL Science and particle size in Armstrong unit was used for the analysis. A Shimadzu analytical balance Model No. AP225WD (manufactured in Switzerland) and a sonicator (Aczet, Made in India) were employed for sample preparation.

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Table 1: Chromatographic conditions

Column	DB-wax 30m, 0.53mm, 0.5µ or equivalent
Injector Temperature	120.0°C
Detector temperature	260.0°C
Flow Control Mode	Pressure
Pressure	3.3 psi
Total Flow	12.6 mL/ min
Column Flow	4.80 mL/ min
Linear Velocity	35.4cm/sec
Split Ratio	1.0
Purge Flow	3.0 mL/minute
Hydrogen Flow	40 mL/minute
Zero Air Flow	400 mL/minute
Run Time	19.00 min
Column Temperature Program	
Rate(℃/min)	--
	15.0
Temperature(℃)	50.0
	230.0
Hold Time(min)	4.00
	3.00

Standard preparation

Accurately weigh 50 mg of each standard—1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridine—into separate 100 mL volumetric flasks containing 10 mL of diluent. Mix the contents and dilute to the mark with the diluent. Next, transfer 1.0 mL of this solution into a 100 mL volumetric flask, dilute to the mark with diluent, and mix thoroughly. Then, dilute 5 mL of this solution into a 25 mL volumetric flask, bring it to the mark with diluent, and mix well. Finally, transfer the prepared standard solution into a GC vial and inject it into the GC system. Weighed accurately 50 mg of each of these,1,3-Dichloro propane, 3-chloro-1-bromopropane, 2-Chloro pyridine standard into an individual 100 mL volumetric flask containing 10 ml diluents, mixand make up the volume. Transfer accurately 1.0 mL of this solution into a 100 mL volumetric flask &make it up to mark with diluent and mix well.Further, dilute 5 ml of this solution into a 25 mL volumetric flask &make it up to mark with diluent, and mix well.The standard was then transferred into aGC vial and injected into the GC system.

Sample preparation

Accurate weighed quatity (2000 mg) of the sample was taken in the centrifuge tube and added about 5 ml of diluent into the same centrifuge tube and shook well. Centrifuge the same for about 5 minutes. The supernatant solution of the sample was transferred directly into the GC vial and injected into the GC system. Chromatographic conditions are shown in Table 1 and chromatogram of blank, reference standard & sample are shown in Fig. 1.

Method validation

Linearityandrange

In the linear regression analysis, linearity was assessed using the correlation coefficient. Six different concentrations from 0.3 µg/ml to 2 µg/mlwere analyzed to evaluate the linearity of the response. The calibration curve was generated based on the relationship between peak area and concentration.

Precision

Repeatability

System precision analysis was done with a standard of 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridine prepared at its test concentration of 1µg/mland chromatograph in six replicates and calculated peak area of all with % RSD. Method precision was evaluated by analyzing six separate preparations of the standard.

Intraday and Interday Precision

It was determined by analysing standard reference standard of 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridineprepared solutions (0.8µg/ml, 0.7µg/ml and 0.8µg/ml) at three different times and the same time.

Accuracy

The accuracy of the method depend on % recovery. The sample solution of synthetic combination were taken in eighteen 10 ml volumetric flasks. In this sample, a standard solution of 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridinewas spiked at four levels (50, 100, 120, and 200%).

Robustness

Tiny meaningful changes in instrumental parameters like rate of flow, were used to evaluate the robustness of the method by injecting of standard solution of (concentration) 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridineand evaluating its effect by peak area measuring and calculated % RSD.

Specificity

Table 2: Calibration curve of 2-Chloro propane

Conc(µg/ml)	0.30	0.50	0.80	1.01	1.21	1.51	2.01
AreaMean± SD	5701	9852	16364	21869	24965	31395	43994

Table 3: Calibration curve of 3-chloro-1-bromopropane

Conc(µg/ml)	0.30	0.50	0.80	1.01	1.21	1.51	2.01
AreaMean± SD	3502	7648	12132	15645	18076	22435	31220

Table 4: Calibration curve of 2 – Chloro pyridine

Conc(µg/ml)	0.30	0.51	0.81	1.01	1.22	1.52	2.03
AreaMean± SD	10586	17880	27382	33810	40947	50594	68882

The method's specificity was confirmed by examining the resolution factors of 1,3-Dichloro propane, 1-bromo-3-chloro propane, and 2-chloro pyridine peaks from the closest adjacent peaks, as well as between all other detected peaks.

RESULT AND DISCUSSION

Linearity and Range

This method demonstrates excellent linearity and a wide linear range, with a linear regression fit of R^2 = 0.9949 and the regression equation, y = 322697.5244x +337.0118. (Table 1) The method has shown strict linearity within the concentration range of 0.3-2.01 µg/ml. Additionally, the plot of residuals reveals a random distribution about zero.Concentration and Area mean±SD for2-Chloro propane (Fig.2.), 3-chloro-1-bromopropane (Fig.3), 2 – Chloro pyridine (Fig. 4) are shown in table 2, 3 and 4 respectively. The method demonstrates excellent linearity and a wide linear range, with a linear regression fit of R^2 = 0.9949 and the regression equation, y = 322697.5244x + 337.0118. (Table 1) The method has shown strict linearity within the concentration range of 0.3-2.01 µg/ml. Additionally, the plot of residuals reveals a random distribution about zero.

Precision

In system precision analysis, % relative standard deviation of the peak area of 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridineobtained for six

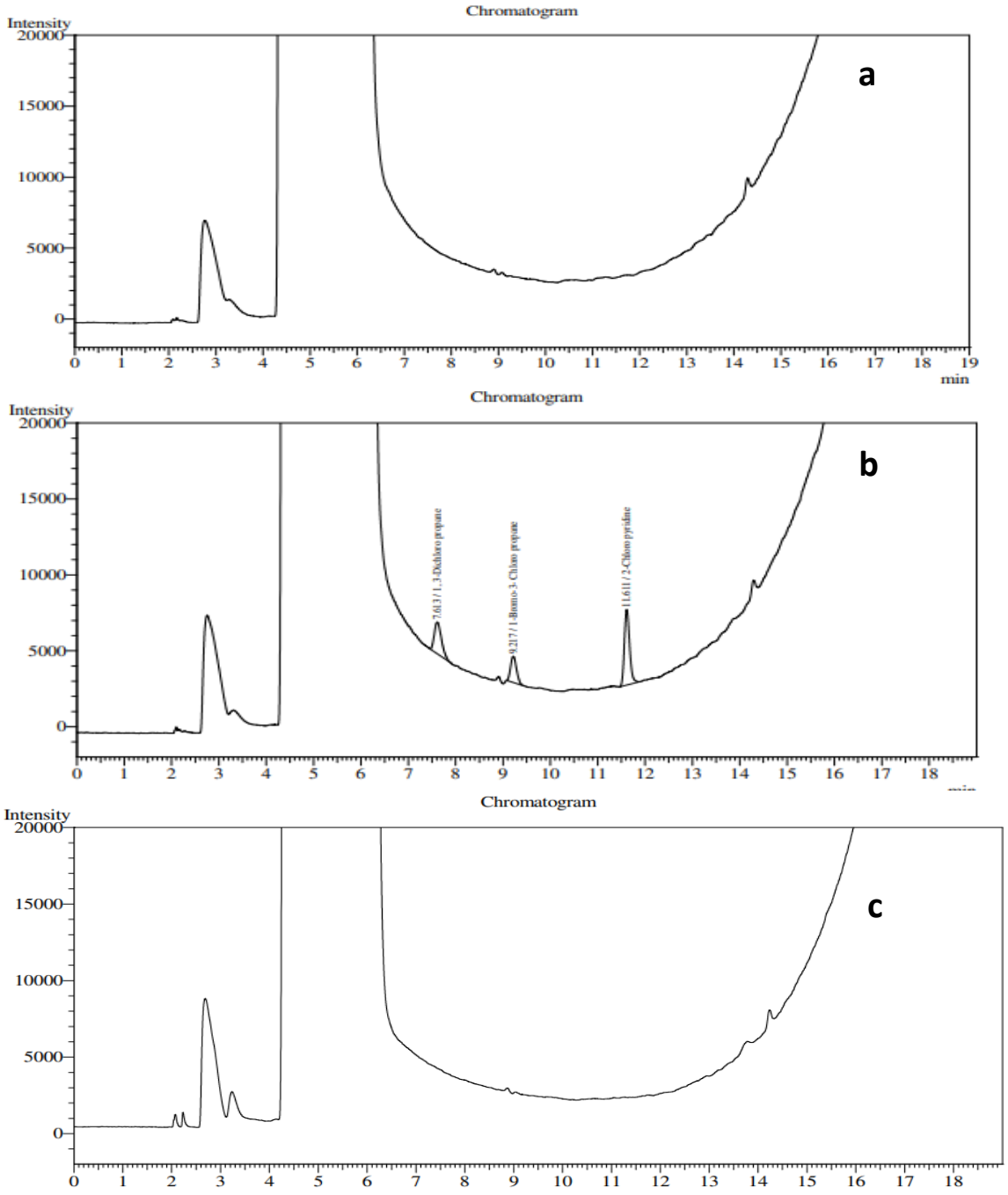

Figure 1: Chromatograms of a:blank, b:reference standard and c:sample.

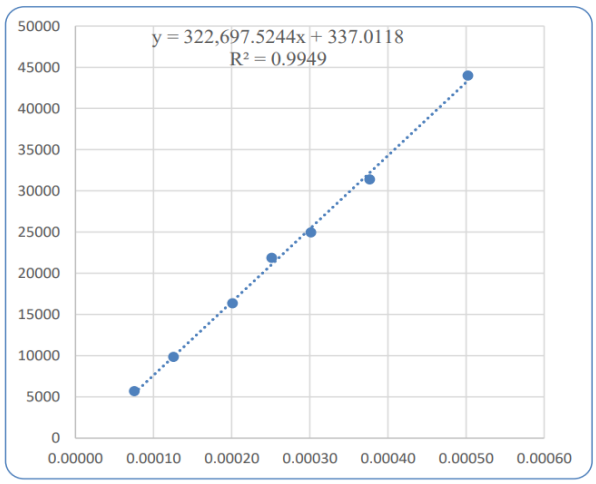

Figure 2: Calibrationcurve of 2-Chloro propane

1-Bromo-3- Chloro propane

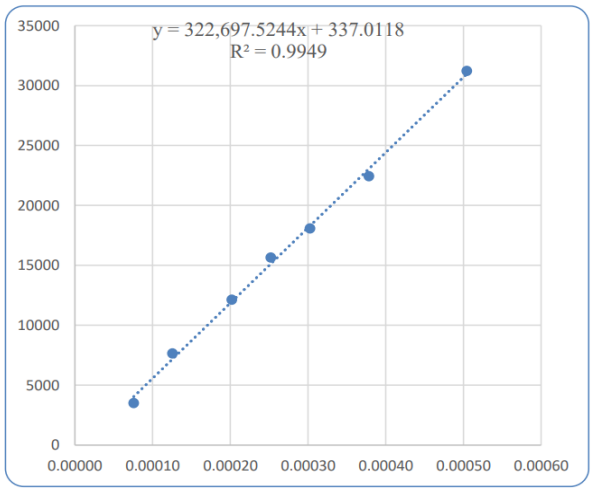

Figure 3: Calibrationcurve of 1- Bromo-3-Chloro propane

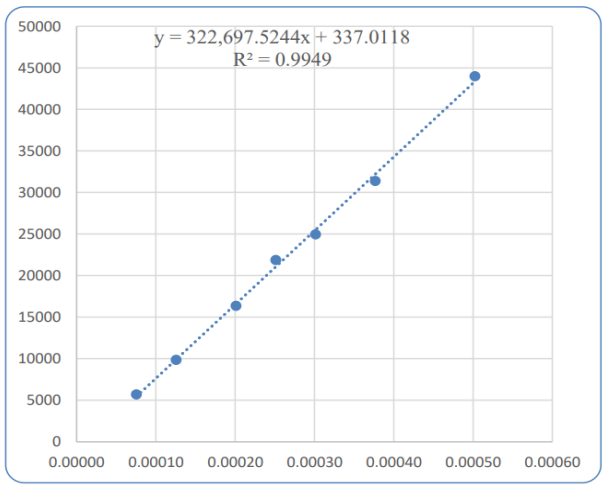

Figure 4: Calibration curve of 1- Bromo-3-Chloro propane

replicate injections was 2.62%, 2.22% and 1,93% respectively. Data is shown in Table 5, table 6 and table 7 for 1,3 Dichloropropane, 3-chloro-1-bromopropane, Precision data of 2-Chloro pyridine respectively.

Table 5: Precision data of 1, 3 Dichloro propane.

Trial	Peak Area	Intraday Precision	
1	21974	Time	Area (Mean±SD)(n=1)
2	22088	0 hr	21661 ± 87.78
3	21023	11 hr	21579 ± 32.57
4	21037	24 hr	22351 ± 22.81
5	21793	Interaday Precision	
6	22397		
Mean	21719		
SD	569	-	22350 ± 428.25
%RSD	2.62%		

Table 6: Precision data of 3-chloro-1-bromopropane.

Trial	Peak Area	Intraday Precision		
1	14872	Time	Area (Mean±SD)(n=3)	%RSD
2	14953	0 hr	14886± 87.78	2.23
3	15151	11 hr	15080 ± 32.57	2.07
4	15818	24 hr	17547 ± 22.81	5.96
5	15354	Interaday Precision		
6	15258			
Mean	15234			
SD	339	-	17936 ± 428.25	6.83
%RSD	2.22%			

Table 7: Precision data of 2-Chloro pyridine.

Trial	Peak Area	Intraday Precision		
1	37996	Time	Area (Mean±SD)(n=3)	%RSD
2	36639	0 hr	36269 ± 87.78	1.84
3	36843	11 hr	36584 ± 32.57	1.77
4	36536	24 hr	34533 ± 22.81	2.92
5	36887	Interaday Precision		
6	35772			
Mean	36774			
SD	710	-	34987 ± 428.25	2.56
%RSD	1.93%			

Accuracy

The accuracy of measuring 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridinewere assessed by preparing five sample solutions containing the compound at the Limit of Quantification (LOQ), which corresponds to 50 percent, and 3 sample solutions containing the compound at levels of 100%, 120%, and 200% of the specified limit. Three measurements were performed for each solution. The

Table 8: Accuracy data of 1, 3 Dichloro propane.

% Level of spike	Wt. of sample in (µg/ml)	Amount of std. added(µg/ml)	Amount added (In %)	Totalamount of std. found (µg). Mean ± SD (n=3)	% Recovery
0	-	-	0	0	0
LOQ	2000.25	0.001	0.0001	5472	84.0
50	2000.19	0.001	0.0001	10221.66	94.1
100	2000.27	0.001	0.0003	22683.33	104.42
120	2000.15	0.001	0.0003	26195.33	100.5
200	2000.31	0.001	0.0005	44289	101.9

Table 9: Accuracy data of 3-chloro-1-bromopropane.

% Level of spike	Wt. of sample in (µg/ ml)	Amount of std. added(µg/ml)	Amount added (In %)	Total amount of std. found (µg). Mean ± SD (n=3)	% Recovery
0	-	-	0	0	0
LOQ	2000.25	0.001	0.0001	5435.33	118.9
50	2000.19	0.001	0.0001	8283.66	108.7
100	2000.27	0.001	0.0003	17216	112.99
120	2000.15	0.001	0.0003	20379.66	111.5
200	2000.31	0.001	0.0005	33225	109.0

Table 10: Accuracy data of 2-Chloro Pyridine.

% Level of spike	Wt. of sample in (µg/ml)	Amount of std. added(µg/ml)	Amount added (In %)	Total amount of std. found (µg). Mean ± SD (n=3)	% Recovery
0	-	-	0	0	0
LOQ	2000.25	0.001	0.0001	10213	92.6
50	2000.19	0.001	0.0001	16757	91.1
100	2000.27	0.001	0.0003	37560.33	102.12
120	2000.15	0.001	0.0003	41539.33	94.1
200	2000.31	0.001	0.0005	68388	93.0

Table 11: LOD ratio

S. No.	Impurity Name	S/ N Ratio
1	1, 3 Dichloro Propane	8.088929
2	3-chloro-1-bromopropane	5.755706
3	2-Chloro Pyridine	12.820438

Table 12: LOQ ratio

S. No.	Impurity Name	S/ N Ratio
1	1, 3 Dichloro Propane	15.278598
2	3-chloro-1-bromopropane	13.139644
3	2-Chloro Pyridine	41.798684

percentage recover for all preparations ranging from 84.0% to 118.9%, are shown in Table 8 for 1,3-Dichloro propane, Table 9 for 3-chloro-1-bromopropaneand and Table 10 for 2-Chloro pyridine.

Limit of quantification and detection

The limits of detection and quantification (LOD & LOQ) were validated with a signal-to-noise ratio of ≥3 &10, respectively.

CONCLUSION

The % assays of the conc. of1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2 – Chloro pyridinewas found in the acceptable range. The % RSD is well within the prescribed limits as per ICH guidelines. The sample preparation is easy and used chemicals are non-toxic. A specific GC method utilizing aDB-wax 30m, 0.53mm, 0.5µ column was adopted to achieve the desired chromatography for the analysis of 1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2 – Chloro pyridine in Trazodone Hydrochloride. The method was thoroughly evaluated for specificity, linearity, accuracy, and robustness to ensure reliable results. The LOD of the method was determined to be 0.8 ppm, 0.7 ppm and 0.8 ppm. This indicates that the method is sensitive enough to detect low concentrations of 1, 3 Dichloro propane, 3-chloro-1-bromopropane, and 2 – Chloro pyridine, making it suitable for analysis in drug substances. Based on above data it can be concluded that the GC method was successfully developed and validated for 1,3-Dichloro propane, 3-chloro-1-bromopropane, and 2-Chloro pyridinecontent in Trazodone Hydrochloride.

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