IJPSR (2026), Volume 17, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 October 2025; received in revised form, 16 December 2025; accepted, 17 December 2025; published 01 April 2026

METHOD DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF GENOTOXIC IMPURITIES IN TRAZODONE HCL DRUG SUBSTANCEUSING UFLC-MS/MS (ESI)

Shankrappa Janiwarad [1, 2], K. R. Ravi Kumar [* 1, 3, 4], P. Venkatesh [2], B. K. Vanitha Devi [1, 3, 4], H. Mallesha [1], B. C. Revanasiddappa [2], R. S. Chandan [2], B. S. Ananya [1] and M. R. Hithesh [1]

DSIR Approved Research and Development Centre [1], Robust Materials Technologies Pvt. Ltd., NGEF Layout, Nagarbhavi, Bangalore - 560072, Karnataka, India.
J. S. S College of Pharmacy [2], Sri Shivarathreeshwara Nagara, Mysuru - 570015, Karnataka, India.
V. R. Institute of Chemical [3], Biological & Information Technology Science, Sri Krishna Complex, 7th Main Road, Bharat Nagar, Bangalore - 560 091, Karnataka, India.
Simposys Healthcare L. L. P., 3rd Floor, Thirumala Complex [4], No.94, NGEF Layout, Naagarabhavi, Bengaluru - 560072, Karnataka, India.

Keywords:

ICH guidelines, Pharmaceutical quality control, Process related impurities, Mass spectrometry, Drug safety assessment

Correspondence to Author:
Dr. K. R. Ravikumar

Chief Executive Officer,
DSIR Approved Research and Development Centre, Robust Materials Technologies Pvt. Ltd., NGEF Layout, Nagarbhavi, Bangalore - 560072, Karnataka, India.

E-mail: ravikumar.kr@robustmaterila.com

ABSTRACT: Due of their ability to damage DNA, genotoxic impurities (GTIs) in active pharmaceutical substances need strict analytical control. Trace process-related GTIs may be present in trazodone hydrochloride, an antidepressant made using multi-step synthetic processes. Nevertheless, there is currently no approved analytical technique for the simultaneous determination of all significant GTIs related to its production. In order to quantify four process-related GTIs in trazodone hydrochloride at sub-ppb levels, a quick and selective UFLC-MS/MS approach was developed and validated. Acetonitrile-5 mM ammonium acetate buffer (pH 5.0, 70:30 v/v) was used for chromatographic separation on an Avantor ACE C18-PFP column (3 µm, 4.6 × 150 mm) under isocratic conditions. Multiple reaction monitoring was used for detection in positive ESI mode. The approach was validated over 0.060-1.200 ppb. For every impurity, linearity was attained with correlation coefficients (R^2) ≥ 0.99. Across three levels, accuracy studies produced recoveries ranging from 79 to 102%. Robustness was verified with intentional changes in flow rate and column temperature, and precision satisfied acceptance standards with %RSD < 15%. When kept in a refrigerator, stock solutions stayed stable for at least three days. All GTIs were found to be below the set regulatory limits when the validated procedure was applied to commercial batches. For routine quality-control monitoring of genotoxic contaminants in trazodone hydrochloride, the established UFLC-MS/MS technique is sensitive, dependable, and appropriate.

INTRODUCTION: Trazodone hydrochloride is a triazolopyridine-derived antidepressant. It was first made available in the 1970s and received US approval in 1978.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.17(4).1178-89

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(4).1178-89

As a serotonin antagonist and reuptake inhibitor (SARI), it is often used for treating major depressive disorders and a number of other off-label diseases, such as fibromyalgia, schizophrenia, chronic pain, insomnia, and symptoms connected to substance abuse [1-6]. Its multi-step synthetic procedure can produce process related

contaminants, like chemicals with potential genotoxicity, despite being clinically efficacious and well tolerated. Due to their potential carcinogenicity and ability to harm the DNA, genotoxic impurities (GTIs) are considered a major toxicological issue.

ICH, EMA, and USFDA regulatory frameworks mandate strict management of GTIs, usually at very low concentration limits, using strategies like daily dose-based thresholds, the ALARP principle, and the Threshold of Toxicological Concern (TTC) of 1.5 µg/day [7-12].

Hence, in order to guarantee the safety of active pharmaceutical ingredients (APIs), effective analytical techniques are crucial. Various analytical methods for trazodone-related contaminants have been documented. Using LC-MS/MS with ppb-level sensitivity, Venugopal *et al.* measured the GTI CCP HCl [13].

A stability-indicating HPLC method. Moreover LC-MS/MS techniques for other contaminants ("Compound A" and "Compound F") have also been reported [14, 15].

However, no published approach quantifies all four primary process-related GTIs generated during trazodone synthesis. By creating and validating a selective UFLC-MS/MS method for the simultaneous quantification of four GTIs- 1-(3-Chlorophenyl) – 4 - (3-chloropropyl) piperazine hydrochloride (CPH), 1-(3-Chloropropyl)-4-(4-chlorophenyl) piperazine (CCP), 1-(3-Chloropropyl)- 4-(3-bromophenyl) piperazine (CBP), and1-(3-Bromopropyl)-4-(3-chlorophenyl) piperazine (BCP) at sub-ppb levels, the study fills this analytical gap and offers a complete tool appropriate for regular quality-control monitoring.

EXPERIMENT:

Chemicals and Reagents: LC–MS grade acetonitrile, methanol, and water were sourced from J.T. Baker (Avantor Performance Materials India Pvt Limited Gujarat, India). LC–MS grade formic acid was obtained from Biosolve (Chimi SARL Netherlands), and glacial acetic acid from (RankemAvantor Performance Materials India Pvt Limited Thane Maharashtra, India). Trazodone hydrochloride API **Fig. 1A** was obtained from Bajaj Healthcare.

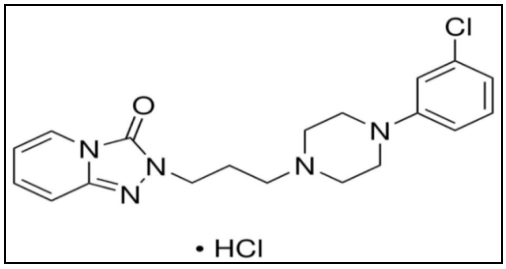

FIG. 1A: MOLECULAR STRUCTURE OF TRAZODONE HCL

The following genotoxic impurities (GTIs) were used:

BCP: 1-(3 - Bromopropyl) – 4 - (3 - chlorophenyl) piperazine **Fig. 1B.**

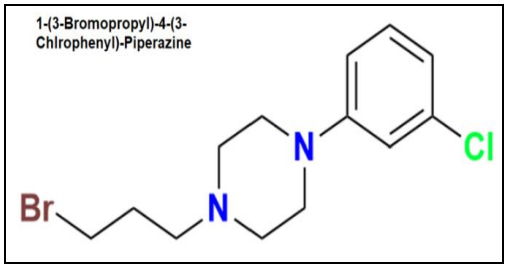

FIG. 1B: MOLECULAR STRUCTURE OF 1-(3-BROMOPROPYL)-4-(3-CHLOROPHENYL)-PIPERAZINE

CBP: 1-(3 - Chloropropyl) – 4 - (3-bromophenyl) piperazine **Fig. 1C.**

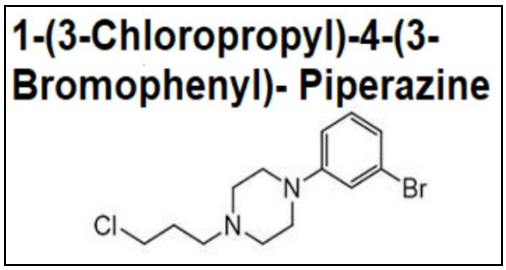

FIG. 1C: MOLECULAR STRUCTURE OF 1-(3-CHLOROPROPYL)-4-(3-BROMOPHENYL)-PIPERAZINE

CCP: 1-(3-Chloropropyl) – 4 - (4-chlorophenyl) piperazine **Fig. 1D.**

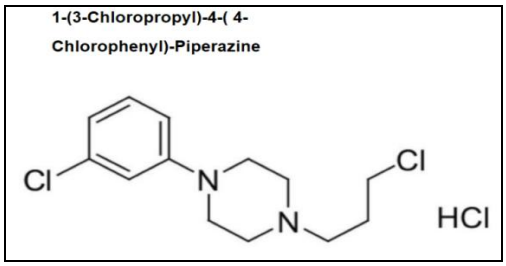

FIG. 1D: MOLECULAR STRUCTURE OF 1-(3-CHLOROPROPYL)-4-(4- CHLOROPHENYL)-PIPERAZINE

CPH: 1-(3-Chlorophenyl) – 4 - (3-chloropropyl) piperazine hydrochloride **Fig. 1E.**

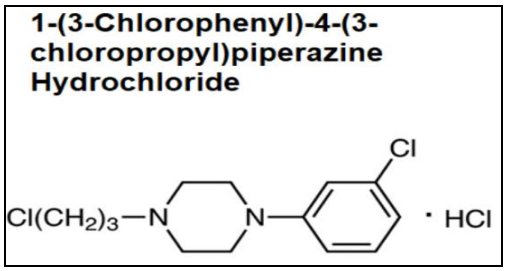

FIG. 1E: MOLECULAR STRUCTURE OF 1-(3-CHLOROPHENYL)-4-(3-CHLOROPROPYL) PIPERAZINE HYDROCHLORIDE

Preparation of Standard Solution: 1mg/ mL of each genotoxic impurity was prepared by dissolving 10mg of impurity in 10 mL LC-MS grade methanol separately. Acetonitrile: 5mM ammonium Acetate pH 5 (70:30); pH of 5mM ammonium Acetate was adjusted with 0.1% glacial acetic acid; was used as the diluent for the preparation of working standard solutions and sample solutions. The individual stock solutions of the impurities mixed to get the system suitability solution with concentration of 0.6 ppb.

Sample Solution Preparation: Trazodone Hydrochloride Sample was prepared at concentration of 1mg/mL (w/v) using the Diluent and injected on the chromatography.

Instrument and Analytical Parameters:

Mass Spectrometry: Analysis was carried out on a Shimadzu LC–MS/MS 8045 triple quadrupole system equipped with a SIL-40C×3 autosampler and LC-40B×3 solvent delivery modules, operating in positive Electrospray Ionization (ESI+) mode. Quantitation was carried out using Multiple Reaction Monitoring (MRM), with instrument parameters optimized to yield the most abundant and selective precursor-to-product ion transitions. Each analyte was identified based on its mass-to-charge (m/z) ratio and characteristic fragmentation pattern, as illustrated in **Fig. 2A** (BCP), **Fig. 2B** (CBP), **Fig. 2C** (CCP), and **Fig. 2D** (CPH). The complete set of optimized source, ionization, and compound-dependent parameters used for MRM acquisition is summarized in **Table 1.**

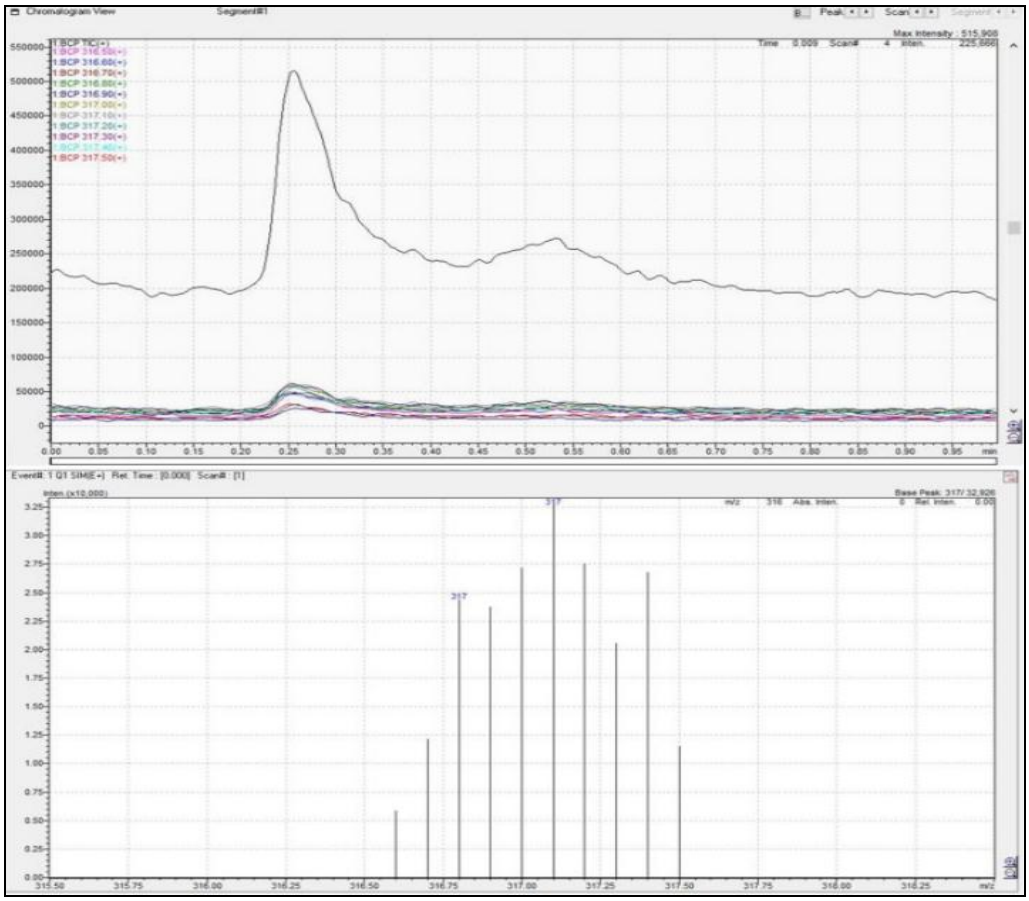

FIG. 2A: MASS SPECTRUM OF PRECURSOR ION FOR 1-(3-BROMOPROPYL)-4-(3-CHLOROPHENYL)-PIPERAZINE (BCP) OBTAINED UNDER OPTIMIZED UFLC–MS/MS CONDITIONS IN POSITIVE ESI MODE

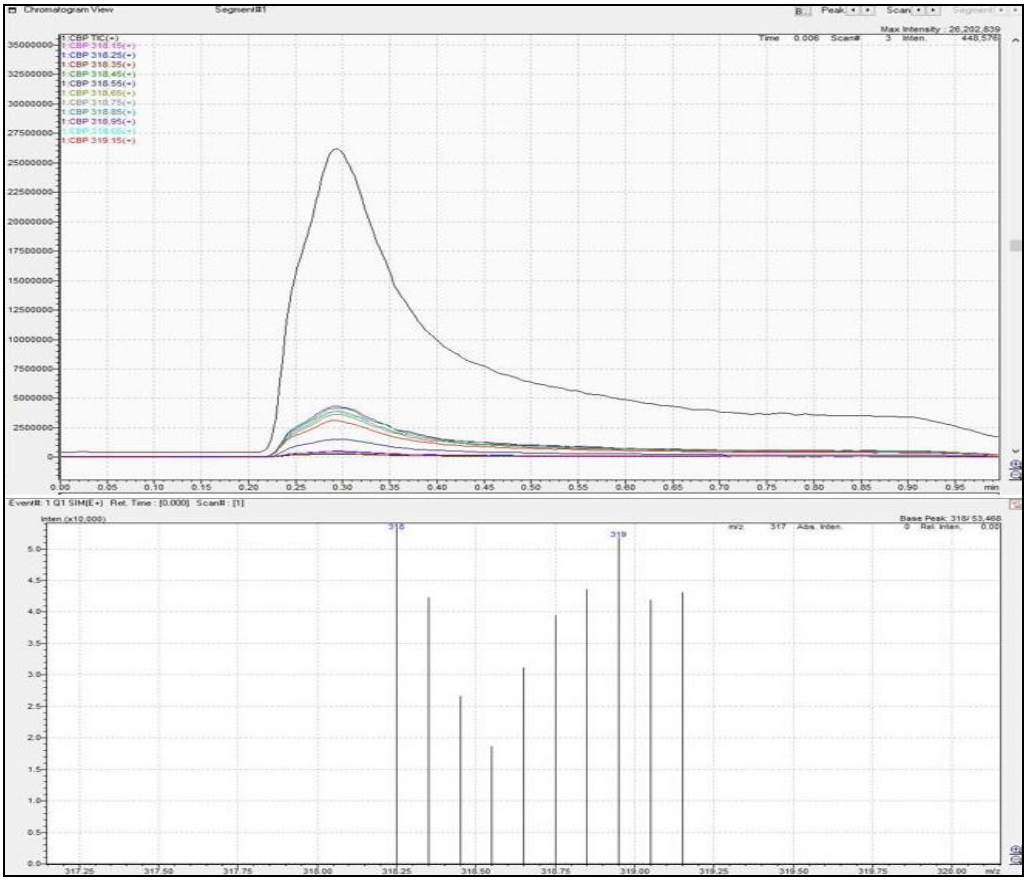

FIG. 2B: MASS SPECTRUM OF PRECURSOR ION FOR 1-(3-CHLOROPROPYL)-4-(3-BROMOPHENYL)-PIPERAZINE (CBP) OBTAINED UNDER OPTIMIZED UFLC–MS/MS CONDITIONS IN POSITIVE ESI MODE.

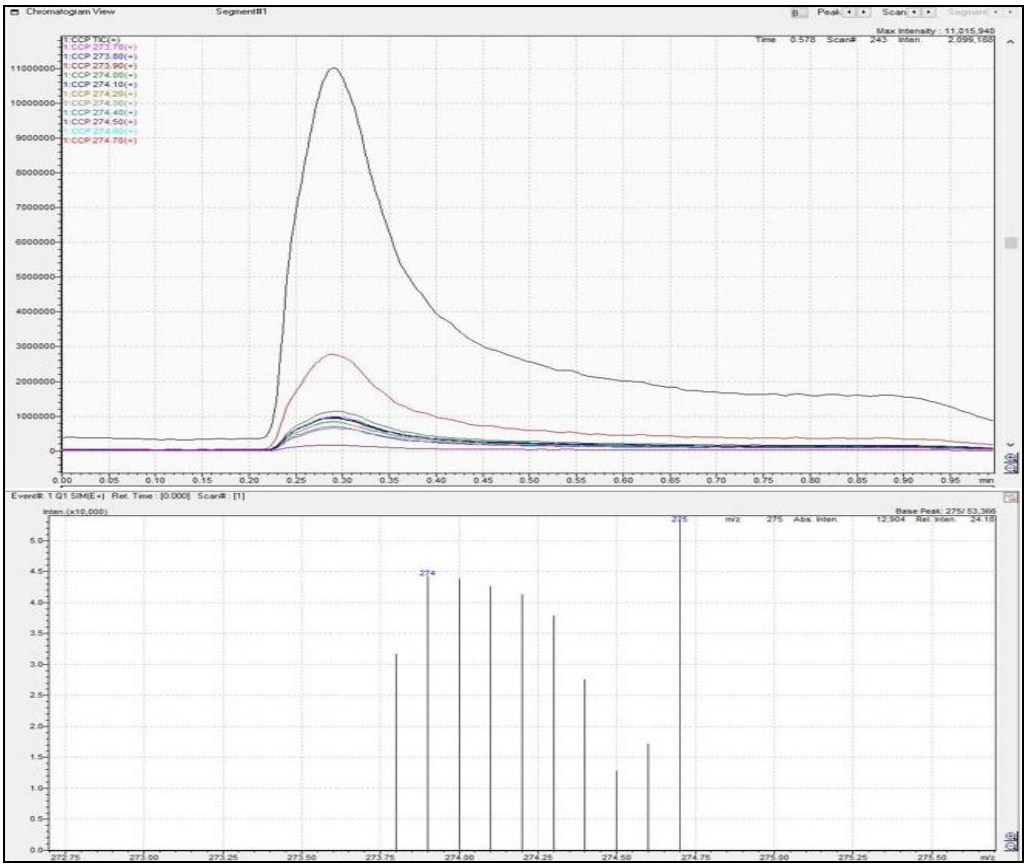

FIG. 2C: MASS SPECTRUM OF PRECURSOR ION FOR 1-(3-CHLOROPROPYL)-4-(4-CHLOROPHENYL)-PIPERAZINE (CCP) OBTAINED UNDER OPTIMIZED UFLC–MS/MS CONDITIONS IN POSITIVE ESI MODE

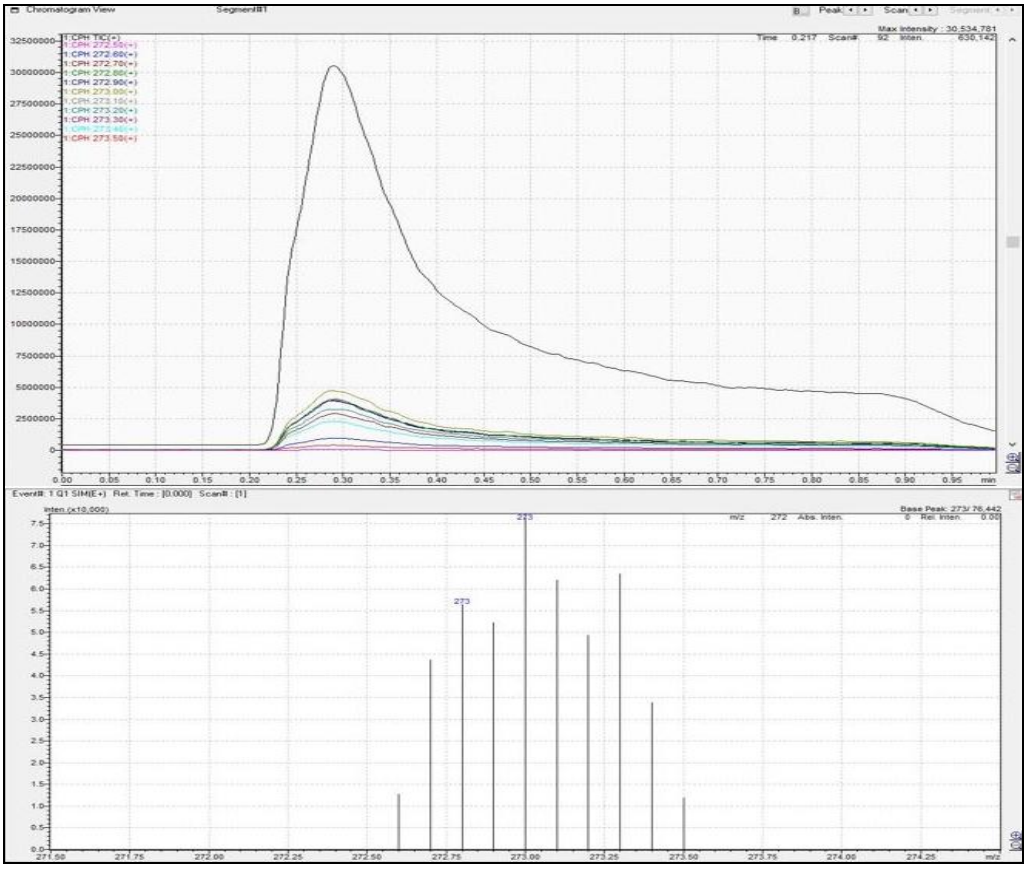

FIG. 2D: MASS SPECTRUM OF PRECURSOR ION FOR 1-(3-CHLOROPHENYL)-4-(3-CHLOROPROPYL)-PIPERAZINE (CPH) OBTAINED UNDER OPTIMIZED UFLC–MS/MS CONDITIONS IN POSITIVE ESI MODE

TABLE 1: MASS SPECTROMETRIC AND UFLC CONDITIONS

Source Parameters	
Interface	ESI
Interface Temperature	300
Desolvation temperature	526
DL Temperature	250
Nebulising Gas Flow	3.00 L/min
Heat Block	400
Drying Gas	On
Drying Gas flow	10.00 L/min

Compound Parameters							
Name Of Genotoxic Impurities	**Precursor m/z**	**Product m/z**	**Dwell Time**	**Q1 Pre Bias**	**Collision Energy**	**Q3 Pre Bias**	**Retention time (RT)**
1-(3-Bromopropyl)-4-(3-Chlorophenyl)-Piperazine	318.15	318.15	100.0	-12.0	--5.0	-22.0	6.19
1-(3-Chloropropyl)-4-(3-Bromophenyl)-Piperazine	318.95	118.10	100.0	-16.0	-46.0	-20.0	6.19
1-(3-Chloropropyl)-4-(4-Chlorophenyl)-Piperazine	273.20	154.15	100.0	-10.0	-27.0	-28.0	5.57
1-(3-Chlorophenyl)-4-(3-chloropropyl)piperazine Hydrochloride	273.20	120.15	100.0	-20.0	-23.0	-22.0	5.57

UFLC Conditions	
Mobile Phase A	5mM ammonium Acetate pH 5
Mobile Phase B	Acetonitrile
Flow Rate	0.8000 mL/min
Injection Volume	20 µL

Autosampler Temperature	5°C
Needle wash Solution	Water: Acetonitrile (30:70 v/v)
Column Temperature	40°C
Run Time	7.00 min
Isocratic Programme	Acetonitrile : 5mM ammonium Acetate pH 5 (70:30)
Diverter Valve	Upto4.00 minutes to Drain

UFLC Conditions: The chromatographic separation was achieved on Avantor ACE (C-18 PFP, 3µm, 4.6*150mm). Acetonitrile: water containing 5mM ammonium Acetate at pH 5 (70:30 v/v) as mobile phase. The UFLC conditions are depicted in **Table 1.**

RESULTS AND DISCUSSION:

Method Development: A systematic literature search was performed for the development of this method. The intention was to develop method for the estimation of Genotoxic Impurities due to which, the reported method for estimation of Trazodone Hydrochloride samples was selected for the preliminary trials of the method development [13]. The experimental conditions were selected by considering the similar physicochemical properties of Trazodone Hydrochloride and genotoxic impurities. The Mass spectrometric optimization was done by performing the auto optimization technique for Genotoxic impurities. Initial trials were found to be promising. Different columns and combination of diluents were tested to improve the peak shape and the retention time of the impurity. During method development, the matrix showed maximum absorbance at 254nm on the Photo Diode Array (PDA) and retention time of 3.490 minutes. According to the method, the eluent was diverted from the column to the drain for the first 4 minutes in order to avoid contamination of the mass spectrometry from higher concentration matrix substances and drugs which are non-targeted analytes. In our optimized method, the eluent was directed to the mass spectrometer after 4 minutes, where the genotoxic impurity eluted, indicating method selectivity as depicted in **Fig. 3**, based on the PDA data.

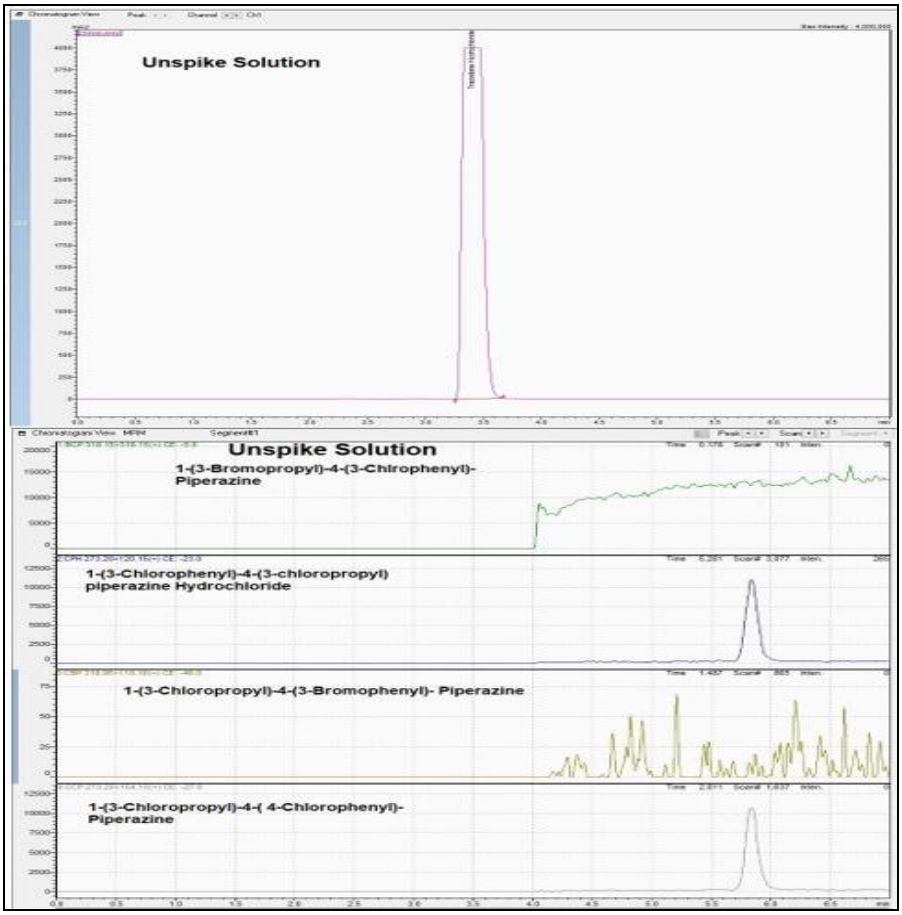

FIG. 3A: CHROMATOGRAMS OF THE UNSPIKED SAMPLE SOLUTION

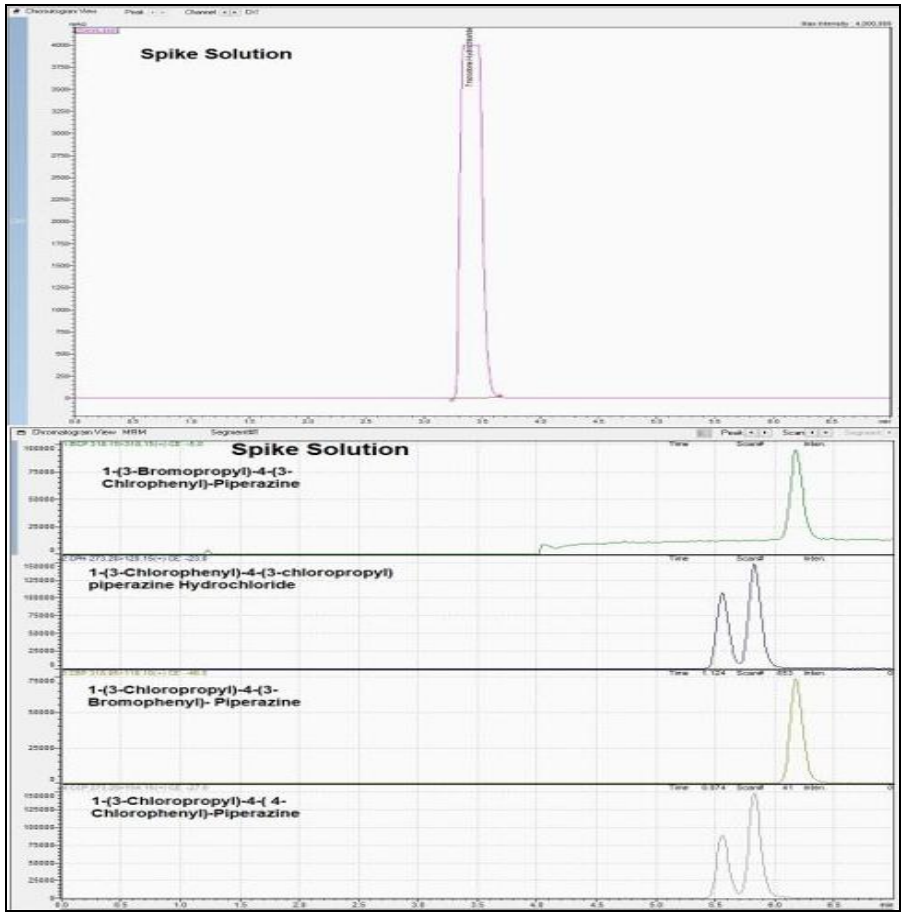

FIG. 3B: CHROMATOGRAMS OF THE SPIKED SAMPLE SOLUTION

Short analysis duration (7.00 minutes) allows the method to be applied for routine quality control tests within a limited time period. For impurities CPH the retention time was found at 5.59 and for CCP at 5.56. It was found that along with these two impurities there was adjacent unknow peak identified at 5.85 in both analytes as shown in **Fig. 3A** and **3B.** These peaks were also observed in the standard solutions and sample solutions except blank. The unknown peaks are found to be well separated from the analyte of interest and have no impact on the recovery of the analytes. For other two impurities i.e. CBP and BCP no unknown peaks observed.

Method Validation: The developed method was validated for the estimation of Genotoxic Impurities in Trazodone Hydrochloride. The method was validatedover the range of 0.060ppb to 1.200ppb for all the impurities, since the limit is same for all the impurities and was evaluated by employing linearity, sensitivity (LOD and LOQ), percent recovery, intra- and inter-day precision, and robustness.

The stock solution was also established for 3 days at refrigerated condition. The results are depicted in **Table 2.**

TABLE 2: VALIDATION SUMMARY RESULTS

Validation Parameter	Sample / Level	Acceptance Criteria	BCP	CBP	CCP	CPH
System Suitability	6 replicate injections at limit (0.06 ppb)	Area %RSD (n=6) ≤ 20%	4.70%	4.18%	12.05%	11.60%
Sensitivity – LOD	7.5% of limit concentration	S/N ≥ 3	37.44	1442.57	236.85	224.72
Sensitivity – LOQ	10% of limit concentration	Average S/N ≥ 10; Area %RSD (n=6) ≤ 20%	23.90 / 8.13%	961.72 / 1.79%	343.76 / 1.57%	294.41 / 1.61%

Selectivity	Single injection of spiked sample at limit	No interference at analyte RT	No interference	No interference	No interference	No interference
Linearity	0.06–1.20 ppb	Correlation coefficient (R^2) ≥ 0.99	0.9992	0.9993	0.9986	0.9992
Accuracy (% Recovery)	Lower: 0.150 ppb		82–92%	85–88%	86–102%	91–100%
	Mid: 0.600 ppb	100 ± 20%	86–89%	87–90%	79–82%	80–83%
	Upper: 0.900 ppb		83–86%	84–86%	95–98%	95–96%
Precision – Repeatability	6 spiked solutions at mid level (0.625 ppm)	%RSD ≤ 15%	4.26%	5.18%	4.98%	5.17%
Precision – Intermediate	Different day / analyst (n=6)	%RSD ≤ 15%	2.19%	4.56%	2.26%	7.30%
	Combined (n=12)	%RSD ≤ 20%	3.24%	4.93%	17.97%	17.61%
Carryover Test	Blank after high standard	Blank ≤ 20% of high standard	No interference	No interference	No interference	No interference
Stock Solution Stability	Stock solution stability was established 3 days	%RSD ≤ 15%	7.19%	5.90%	3.12%	6.25%
Robustness – Low Flow Rate	System suitability solution (n=6)	%RSD ≤ 20%	5.11%	9.38%	6.09%	4.50%
Robustness – High Flow Rate	System suitability solution (n=6)	%RSD ≤ 20%	6.16%	8.91%	3.94%	3.51%
Robustness – Low Column Temp.	System suitability solution (n=6)	%RSD ≤ 20%	6.70%	12.02%	3.54%	4.95%
Robustness – High Column Temp.	System suitability solution (n=6)	%RSD ≤ 20%	4.36%	7.48%	5.99%	9.52%

System Suitability: The system suitability was evaluated by using six replicate injections of the standard solution at 0.600 ppb for all impurities, equivalent to the limit with respect to sample.

Sensitivity (LOD and LOQ): Based on the recommended acceptable daily intake, LOD and LOQ were set at 7.5% and 10% of the daily acceptable limit respectively. Accordingly, the LOD was established at 0.047ppb and LOQ at 0.0625ppb. LOD corresponded to a signal-to-noise(S/N) ratio greater than 3 and LOQ corresponded to a S/N ratio more than 10 with a % RSD for 6 replicate injections of LOQ not exceeding 20%.

Carryover: Carryover test was established to test and measure the unintended transfer of the analyte from one test solution to another. Established by injecting lower and standard solution at 200% level of limit along with inter dispersed blank solutions in the sequence. No interference should be observed at the retention time of analyte in blank solutions, if observed it should be less than 20% of High concentration Standard solution.

Selectivity: The selectivity was established by analysing the sample and spiked sample at limit level to verify the interference at the retention time of the analyte.

The chromatograms of blank, unspiked, and spiked samples **Fig. 3A** and **Fig. 3B** confirmed the absence of interfering peaks at the retention times of BCP, CBP, CCP, and CPH.

The trazodone API peak was also well resolved. The unknown peak at 5.85 min remained baseline separated and did not affect analyte determination

Linearity: A 6-point calibration curve (0.060, 0.150, 0.300, 0.600, 0.900, 1.200ppb) was prepared to establish the linearity in a concentration range of 0.060ppb to 1.200ppb.

The coefficient of determination (R^2) for the Genotoxic Impurities was determined to be greater than 0.99. **Fig. 4A** (BCP), **Fig. 4B** (CBP), **Fig. 4C** (CCP), **Fig. 4D** (CPH). Calibration plots are shown in **Fig. 4A-4D**, each corresponding to one impurity.

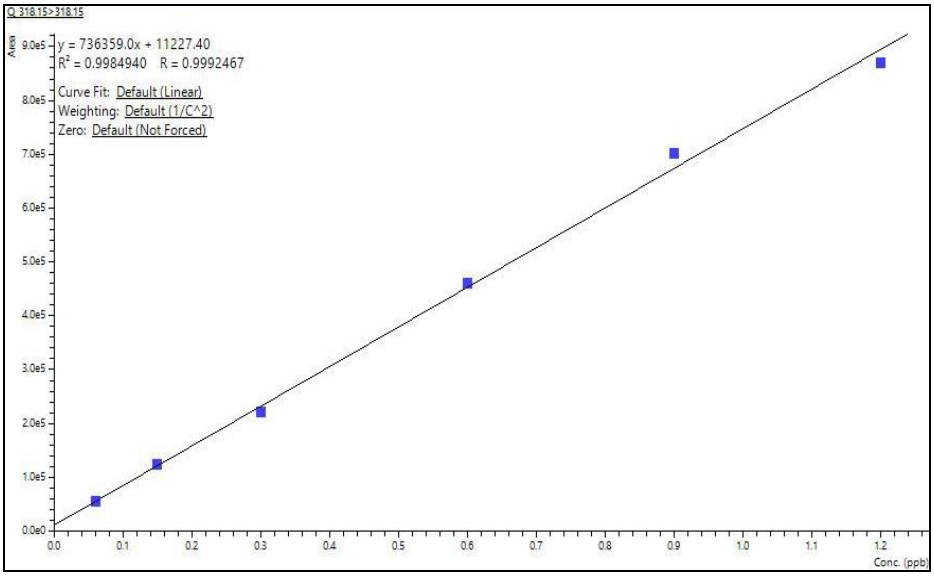

FIG. 4A: CALIBRATION CURVE FOR 1-(3-BROMOPROPYL)-4-(3-CHLOROPHENYL)-PIPERAZINE (BCP)

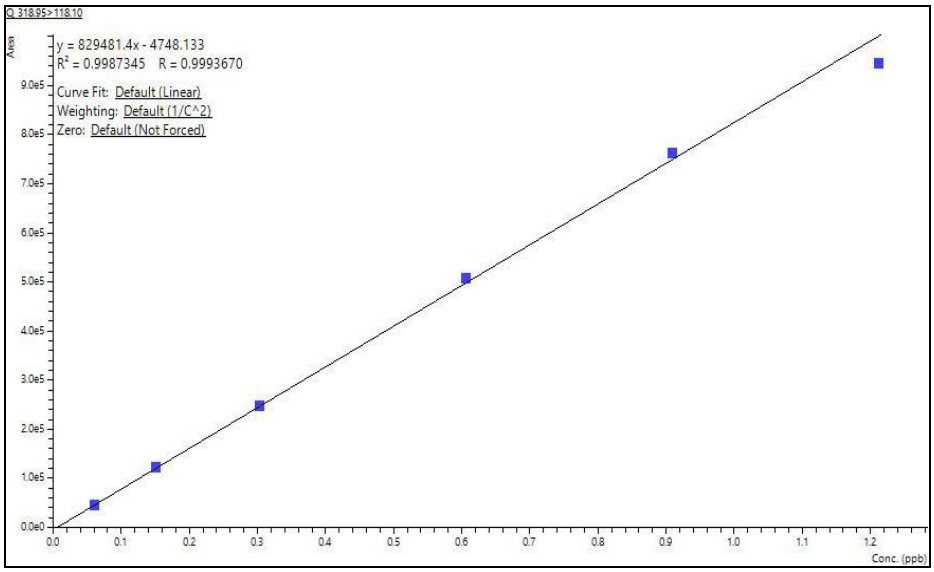

FIG. 4B: CALIBRATION CURVE FOR 1-(3-CHLOROPROPYL)-4-(3-BROMOPHENYL)-PIPERAZINE (CBP)

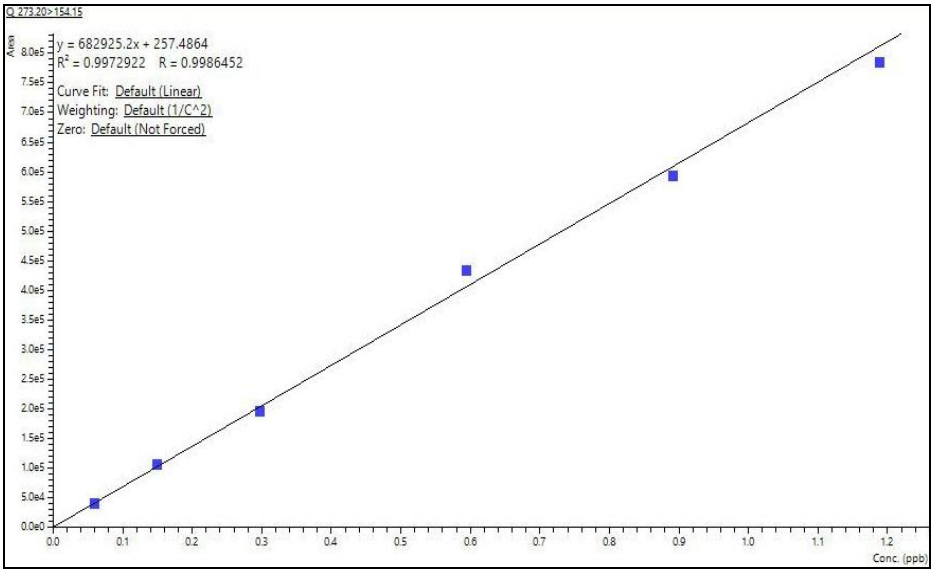

FIG. 4C: CALIBRATION CURVE FOR 1-(3-CHLOROPROPYL)-4-(4-CHLOROPHENYL)-PIPERAZINE (CCP)

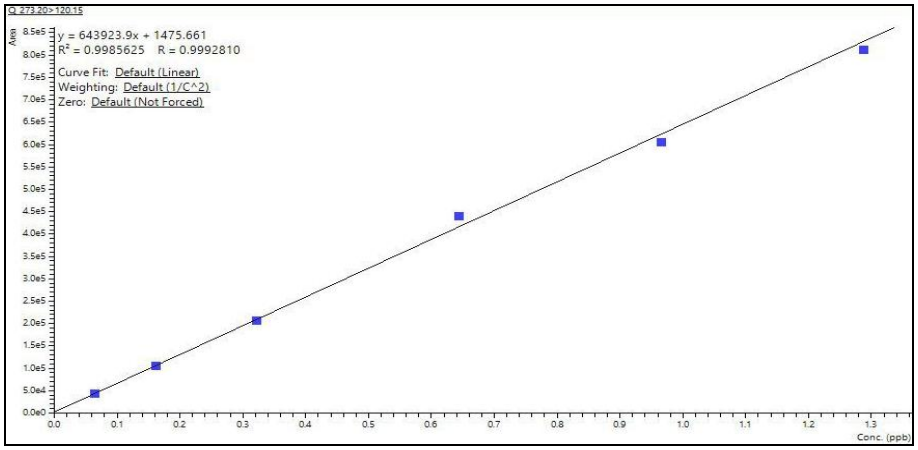

FIG. 4D: CALIBRATION CURVE FOR 1-(3-CHLOROPHENYL)-4-(3-CHLOROPROPYL)-PIPERAZINE (CPH)

Accuracy and Precision: Recovery for the method was evaluated by spiking the sample at 3 different levels (Lower level- 0.150ppb limit-level-0.600ppb and Upper level-0.900ppb) in triplicate. Percent recovery was calculated using the equation:

% Recovery = Concentration obtained / concentration Spiked x 100

The accuracy of the method was confirmed as the recovery of the impurity at each level was within 100±20 %. The precision was established by spiking the sample at the daily acceptable limit (0.625 ppm) in 6 preparations. The observed %RSD for precision was found to be less than 10.0%. Intermediate precision was assessed by a second scientist on a different day using the same instrument. The obtained results from the intermediate precision experiment indicated that the % RSD value of the six samples of genotoxic impurities was less than 15.0 % and the combined % RSD of the 12 solutions was less than 20.0 %.

Robustness: Robustness indicates the ability of the method to remain unaffected by small variations in the method parameters, such as flow rate (with 10% variation in set flow i.e.0.720 -0.880 mL/min) and column temperature (with 5% variation of set value i.e. 35^{o}C-45^{o}C) by injecting 6 replicate injections of the standard solution at 0.600ppb. The %RSD for the analyte remained within the acceptance criteria i.e. NMT 15%.

Stock Solution Stability: Stock solutions of all impurity standards established by analysing the fresh standard solutions against the standard solutions stored at refrigerated temperature for specific period of time. Stock solution stability was established for 3 days at refrigerated temperature.

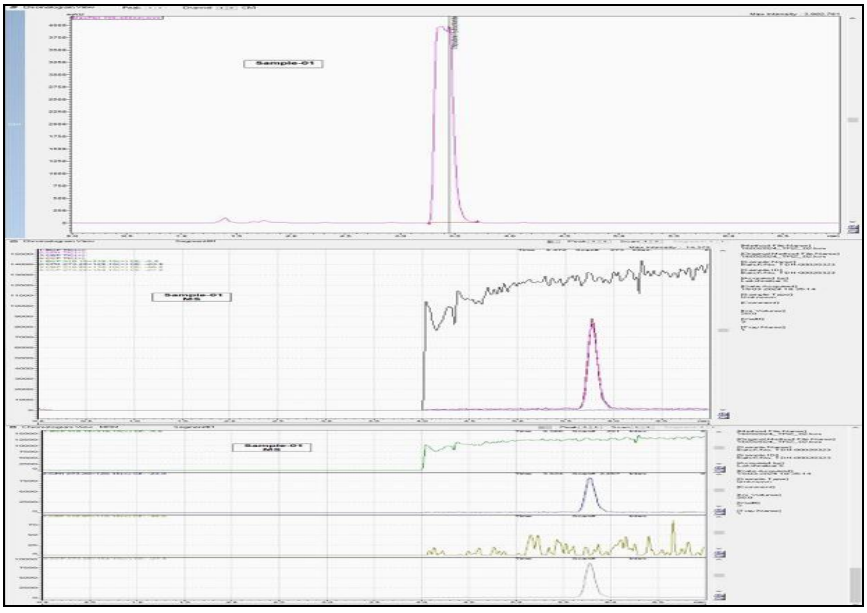

FIG. 5: CHROMATOGRAMS OF COMMERCIAL TRAZODONE HYDROCHLORIDE SAMPLES.

Sample Analysis: The validated method was successfully applied for the estimation of Genotoxic Impurities in commercial samples of Trazodone Hydrochloride. System suitability and bracketing standards were consistent with the acceptance criteria. The Genotoxic Impurities were below the acceptance criteria. The chromatograms of each sample is depicted in **Fig. 5.**

CONCLUSION: The four process-related genotoxic contaminants in trazodone hydrochloride can be monitored at sub-ppb levels using the extremely sensitive and selective UFLC–MS/MS technique. Even in the presence of small degradant peaks, the method consistently showed outstanding analytical performance, including excellent linearity, precision, accuracy, and resilience, guaranteeing dependable quantification. Standard solutions continued to be appropriate for multi-day usage, enabling regular laboratory processes, according to stability evaluations. Method's relevance for quality assessment was highlighted by the analysis of commercial API batches. This confirmed that all contaminants were present well below regulatory limits. The technology ensures adherence to international requirements for genotoxic impurity monitoring and is ideally suited for routine quality-control contexts due to its quick runtime, high repeatability, and capacity to identify impurities at trace concentrations.

ACKNOWLEDGEMENTS: The authors acknowledge Robust Materials Technologies Pvt Ltd., J.S.S College of Pharmacy and VR Unnati Health and Education Trust for access to the resources.

Data Availability: The data supporting this study's findings are available from the corresponding author, upon reasonable request.

CRediT Authorship Contribution Statement: Shankrappa Janiwarad: Writing – original draft, Methodology, Investigation, Conceptualization, Data curation. Dr. K.R. Ravi Kumar, Vanitha Devi B. K., Dr. Mallesha. H: Facilitating the instrumentation, materials and facility to carry out the development and validation. Dr. Venkatesh P, Dr. B.C. Revanasiddappa, Dr. Chandan R S: Visualization, Methodology, Review and Editing, Supervision, Conceptualization, Data curation. Ananya B S, Hithesh M.R: Formal Analysis, Literature search, Writing Conceptualization.

CONFLICT OF INTEREST: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES:

1. Brogden RN, Heel RC, Speight TM and Avery GS: Trazodone. Drugs 1981; 21(6): 401–429.
2. Haria M, Fitton A and McTavish D: Trazodone. Drugs Aging 1994; 4(4): 331–355.
3. Shin JJ and Saadabadi A: Trazodone. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470560/
4. Bossini L, Casolaro I, Koukouna D, Cecchini F and Fagiolini A: Off label uses of trazodone: a review. Expert Opin Pharmacother 2012; 13(12): 1707–1717.
5. Fagiolini A, Comandini A, Dell'Osso MC and Kasper S: Rediscovering trazodone for the treatment of major depressive disorder. CNS Drugs 2012; 26(12): 1033–1049.
6. Khouzam HR: A review of trazodone use in psychiatric and medical conditions. Postgrad Med 2017; 129(1): 140–148.
7. Szekely G, Amores de Sousa MC, Gil M, Castelo Ferreira F and Heggie W: Genotoxic impurities in pharmaceutical manufacturing: sources, regulations, and mitigation. Chem Rev 2015; 115(16): 8182–8229.
8. Soyseven M, Keçili R, Aboul Enein HY and Arli G: Determination of potential genotoxic impurity, 5 amino 2 chloropyridine, in active pharmaceutical ingredient using HPLC UV. J Chromatogr Sci 2020.
9. Raman NVVSS, Prasad AVSS and Reddy KR: Strategies for the identification, control and determination of genotoxic impurities in drug substances. J Pharm Biomed Anal 2011; 55(4): 662–667.
10. Bercu JP, Dobo KL, Gocke E and McGovern TJ: Overview of genotoxic impurities in pharmaceutical development. Int J Toxicol 2009; 28(6): 468–478.
11. CHMP. Guideline on the limits of genotoxic impurities. EMA; 2006. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-limits-genotoxic-impurities_en.pdf
12. McGovern T and Jacobson Kram D: Regulation of genotoxic and carcinogenic impurities in drug substances and products. TrAC Trends Anal Chem 2006; 25(8): 790–795.
13. Venugopal N, Reddy AVB, Madhavi G, Jaafar J, Madhavi V and Reddy KG: Trace level quantification of 1 (3 chloropropyl) 4 (3 chlorophenyl) piperazine HCl genotoxic impurity in trazodone using LC–MS/MS. Arab J Chem 2019; 12(7): 1615–1622.
14. Goel V: Quantification and confirmation of impurities in trazodone API for quality control by LC–MS/MS in accordance with pharmacopeia guidelines. ASMS Conference 2018.
15. ProQuest. 2025. Available from: https://www.proquest.com/openview/a75722334f0f103b545904a0588dd640/

How to cite this article:
Janiwarad S, Kumar KRR, Venkatesh P, Devi BKV, Mallesha H, Revanasiddappa BC, Chandan RS, Ananya BS and Hithesh MR: Method development and validation of analytical method for estimation of genotoxic impurities in trazodone HCl drug substance using UFLC-MS/MS (ESI). Int J Pharm Sci & Res 2026; 17(4): 1178-89. doi: 10.13040/IJPSR.0975-8232.17(4).1178-89.