ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 3, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

TRACE LEVEL QUANTIFICATION OF C_1-C_3 ALKYL CHLORIDE GENOTOXIC IMPURITIES IN TRIMETAZIDINE DI HYDROCHLORIDE DRUG SUBSTANCE USING STATIC HEADSPACE GAS CHROMATOGRAPHY

RAJAVENKATA PRASAD PATHA[1,2]*, KARUNAKAR DASA[3], RAMA DEVI BHOOMIREDDY[1], RAVI KIRAN PANCHAKARLA[2], RAJ KUMAR KOKKONDA[2]

[1]Department of Chemistry, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India. [2]Analytical Research and Development, Sai Life Sciences Limited, Genome Valley, Hyderabad, Telangana, India. [3]Department of Chemistry, Government Degree College, Kukatpally, Telangana, India.
***Corresponding author: Rajavenkata Prasad Patha; Email: bageerath.p@gmail.com**

Received: 1 January 2025, Revised and Accepted: 14 February 2025

ABSTRACT

Objectives: The objective of this study was to develop and validate a gas chromatography-headspace (GC-HS) method for the quantification of genotoxic alkyl chloride impurities (chloromethane, ethyl chloride, and isopropyl chloride) in trimetazidine dihydrochloride, ensuring compliance with International Council for Harmonization M7 guidelines.

Methods: A GC-HS method was optimized using a DB-1 column (60 m×0.32 mm, 3.0 µm) with nitrogen as the carrier gas. Key parameters included HS conditions with an oven temperature of 95°C, sample line temperature of 105°C, and transfer line temperature of 115°C, along with a split ratio of 1:10 and a flow rate of 10.2 psi. The oven temperature program was set to start at 40°C for 15 min, followed by an increase of 30°C/min to 250°C, held for 15 min. Method validation assessed linearity, detection limits, quantification limits, accuracy, precision, and solution stability.

Results: The method exhibited excellent linearity (r^2>0.999), low limits of detection (0.6 ppm) and quantification (1.8 ppm), and high accuracy (91.0–114.0% recovery). Precision was confirmed with relative standard deviations below 5%. Sample solutions remained stable for up to 48 h, demonstrating the method's robustness and reliability for routine analysis.

Conclusion: The developed GC-HS method is a robust, accurate, and regulatory-compliant approach for the trace-level quantification of genotoxic alkyl chloride impurities in trimetazidine dihydrochloride, ensuring the safety and quality of the pharmaceutical product.

Keywords: Genotoxic impurities, Gas chromatography, Method validation, Chloroalkanes, Drug substance.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i3.53750. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Trimetazidine dihydrochloride (TMZ) is a cytoprotective anti-ischemic molecule, which enhances heart muscle function using glucose as an energy inhibiting the use of fatty acid metabolism [1-3]. The structure of TMZ is presented in Fig. 1a, is mostly used for the treatment of angina pectoris, and in some cases used for the treatment of tinnitus and dizziness. It is declared as a prohibited substances list under the category of hormone and metabolic modulators by world anti-doping agency [4]. Synthesis of active pharmaceutical ingredients (APIs) involves multiple chemical conversions where there could be a possibility of formation of potential genotoxic impurities (PGIs) with no therapeutic benefit but create damage to the genetic material of the cell. Hence, regulatory agencies across the globe are insisting pharmaceutical manufactures perform risk assessment and establish control strategies to avoid the formation of PGIs [5].

Typically, alcoholic hydrochloric or hydrobromic acid solutions were used for the salt formation of the APIs consisting of either primary or secondary amine to have better physicochemical properties, enhance solubility in aqueous systems and bioavailability of the drug in the human body. As presented in Fig. 1b, the use of lower chain alcohols in the synthetic process could lead to the formation of respective chloroalkane impurity and can readily react with nucleic acid bases to cause mutation, leading to the formation of the malignant [6]. During the synthesis of TMZ, methanolic hydrochloric acid was employed in the salt formation step, ethanol and isopropanol were used in the preparation of precursor intermediates. According to the structural alerts classification [7,8], chloroalkanes are categorized as alkylating agents among genotoxic impurities, and trace residues may be present as impurities in TMZ. Many regulatory agencies across the globe postulated guidelines for the control of PGI's in pharmaceutical products [9-11]. The International Council for Harmonization (ICH) guidelines provide a framework for ensuring both safety and quality by establishing acceptable limits that minimize risk to patients [11]. Given TMZ's maximum daily dose of 80 mg/day and a permitted daily exposure of 1.5 µg/day, the individual limit for C1-C3 chloroalkanes was determined at 18.75 ppm.

Alkyl chlorides being volatile and ultra-violet inactive quantification using the liquid chromatography would be difficult for the direct determination, whereas quantification using auto-liquid injection GC poses the challenge of matrix interference and high loading of APIs will occur on the column resulting in the fast deterioration of the column [12,13]. To overcome these challenges, static headspace (HS) sampler technique was employed in the present study. Literature survey revealed that there are some published procedures for the trace quantification of chloroalkanes in the variety of sample matrices [14-17]. Published methods applied either complex derivatization process or used auto-liquid injection mode for the analysis, in the present study developed a superior method with better sensitivity and simultaneous quantification of multiple short chain chloroalkane impurities. The finalized method validated as per the current regulatory guidelines.

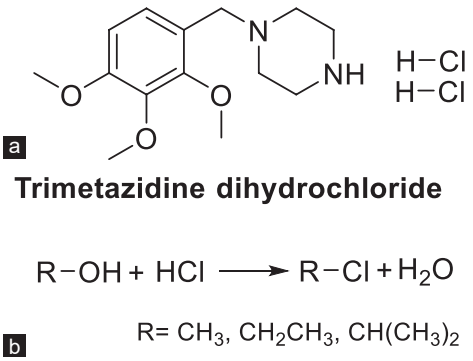


Fig. 1: (a) Structure of trimetazidine dihydrochloride and (b) synthetic scheme for the formation of C1-C3 chloroalkane

The quantification of genotoxic impurities in drug substances has gained significant importance in recent times due to the critical need for ensuring human health and safety. Specifically, alkyl chloride impurities, which are generated throughout the synthetic process from raw materials to final formulation products, pose a concern [18]. In addition, the presence of chlorides in water can become entrapped in the crystalline lattice of products, subsequently interacting with alcohols during stability studies, thereby compromising the robustness and quality of the drug substance [19]. The developed and validated method, utilizing static HS gas chromatography (GC), provides a reliable approach for the online monitoring of chloroalkane impurities in water samples, solvents, drug substances, and drug products. The objective of this method is to enhance the detection and quantification of C1-C3 chloroalkane impurities, ensuring the safety and quality of pharmaceutical products. To the best of current knowledge, no prior publications have addressed the presence of C1-C3 chloroalkane impurities in TMZ drug substances and products.

METHODS

Chemicals and reagents

Chloromethane, ethyl chloride, isopropyl chloride, and 1-methyl-2-pyrrolidinone (NMP) were procured from Merck (Mumbai, India). Rakshith Drugs, Hyderabad, India, gifted trimetazidine dihydrochloride, and GC-grade nitrogen (N2), hydrogen, along with zero-air, were procured from Siddi Vinayaka Industrial Gases Private Limited, based in Hyderabad, India. DB-1 column (60 m×0.32 mm, film thickness 3.0 µm) was procured from Agilent Inc. USA.

Instrumentation and GC conditions

A Shimadzu GC-2010 system, equipped with a flame ionization detector (FID), HS autosampler model HS-20, and an Empower data handling system, was employed for this study. Method development trials were conducted using various stationary phase chemistries and polarities. The final optimized chromatographic conditions utilized a DB-1 column with a stationary phase of 100% dimethylpolysiloxane (60 m×0.32 mm i.d.×3.0 µm film thickness). Nitrogen served as the carrier gas. The oven temperature program initiated at 40°C, held for 15 min, followed by a ramp of 30°C/min–250°C, where it was maintained for 15 min.

The injector temperature was set at 180°C, with the GC system operating in pressure flow control mode at 10.2 psi, and a split ratio of 1:10. The detector temperature was maintained at 250°C. Nitrogen was used as the makeup gas at a flow rate of 30 mL/min, while hydrogen and air were supplied at 40 mL/min and 400 mL/min, respectively.

The HS conditions were as follows: The oven temperature was set at 95°C, the sample line temperature at 105°C, and the transfer line temperature at 115°C. Vial agitation was conducted at level 3. The gas pressure was maintained at 10.00 psi, with an equilibration time of 30.00 min. Pressurization and pressure equilibration times were 2.00 min and 0.20 min, respectively. The sample load time was set to 2.00 min, with a load equilibration time of 0.10 min, and an injection time of 1.00 min. These parameters were meticulously optimized to facilitate accurate and efficient sample analysis.

Preparation of standard and sample solutions

Primary stock solutions of each alkyl chloride were prepared at a concentration of 1.0 mg/mL by purging the respective gases from cylinders into volumetric flasks containing N-methyl-2-pyrrolidone (NMP). These primary stock solutions were subsequently diluted to produce secondary stock solutions at a concentration of 18.0 µg/mL for each alkyl chloride. Further dilutions of both primary and secondary stock solutions were performed with NMP to prepare working solutions for system suitability tests, calibration curve standards, and accuracy assessments. Test sample solutions of temozolomide (TMZ) were prepared at a concentration of 100 mg/mL in NMP. A 1.0 mL aliquot of each prepared solution was transferred into a 20 mL HS vial for analysis, following the established experimental conditions.

Method validation

The developed method was validated in accordance with the regulatory guidelines [16,17]. The following validation parameters were assessed: Sensitivity, selectivity, accuracy, precision, linearity, robustness, and solution stability [20].

Linearity

To establish the linearity of the method for each alkyl chloride, six concentrations ranging from 1.8 to 27.8 ppm were prepared and analyzed. Calibration curves were constructed using least-squares regression analysis. Statistical evaluation of the calibration data, including the calculation of correlation coefficients (R^2), was performed to confirm that the method exhibits a linear response across the specified concentration range. This analysis ensured the method's reliability and accuracy in quantifying alkyl chlorides within the defined range [20].

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for veratryl chloride were determined using the HS GC-FID system by analyzing the HS of solutions containing veratryl chloride at concentrations between 0.2 and 2.0 ppm. The LOD and LOQ were calculated based on signal-to-noise (S/N) ratios of 3:1 and 10:1, respectively [18].

Accuracy and precision

Accuracy and precision were assessed by analyzing the percentage recovery and relative standard deviation (RSD) for each alkyl chloride. Triplicate determinations were performed at four concentration levels, ranging from the LOQ to 200% of the specification limit. Both unspiked and spiked samples were evaluated to ensure comprehensive assessment. The method was deemed accurate if the percentage recovery values fell within 100±20%, and precise if the RSD values were below 10%. These criteria ensured the method's reliability in quantifying alkyl chlorides across the specified concentration range [20].

Solution stability

The stability of the spiked sample solutions was evaluated by storing the solutions at room temperature for various time intervals. Stability was confirmed by monitoring changes in analyte concentration over time, using the initial concentration as a reference. Any significant deviations in analyte concentration were analyzed to ensure the method's reliability for extended storage periods under specified conditions [20].

Handling precautions

All the alkyl halides are genotoxic and lachrymatory substances; hence, appropriate safety precautions such as personal protective equipment and laminar flow-controlled fume hood were used for the preparation of sample and standard solutions.

RESULTS

Method development and optimization of HS and GC conditions

For the trace level quantification of PGI's, selection of right analytical technique based on the property and the target specification of analyte(s) is critical step in the method development [18]. GC separation mode coupled with HS sampler is a key analytical tool for quantifying impurities that are volatile [17-19]. Based on the target limit of 18.75 ppm for the respective alkyl chloride, initial method development experiments were performed by screening various stationary phases with different polarities such as DB-624 (6% cyanopropyl-phenyl), DB-1701 (14% cyanopropyl-phenyl), DB-Wax (polyethylene glycol), DB-5 (5% phenyl), and DB-1 (100% dimethylpolysiloxane) using auto-liquid injection. Good resolution for the alkyl chlorides achieved on DB-1 column, but matrix interference was observed due to introduction of high concentration of TMZ and detrimental effect on the peak shape of the alkyl halides. Hence, static HS option was explored to avoid the matrix effect on the quantification. Other experimental parameters such as HS parameters, flow rate, split ratio, and oven temperature program were investigated to optimize the sensitivity of the target analytes. Finalized method conditions are presented in Table 1. With the change in the flow rate and oven temperature program, though there is change in the retention time no significant increase the separation on DB-1 column. However, significant change in the response was observed with the change in the split ratio. A representative stack overlay chromatogram of NMP, system suitability standard, and TMZ sample is shown in Figs. 2a-c.

Method validation

Specificity

The specificity study, as shown in Fig. 3, revealed that methyl chloride, ethyl chloride, and isopropyl chloride eluted at retention times of 7.36, 10.33, and 14.27 min, respectively. No significant interference was observed at the retention times of the analytes when a blank injection was analyzed, confirming the specificity of the method for the target alkyl chlorides.

LOD and LOQ

The LOD and LOQ values for methyl chloride, ethyl chloride, and isopropyl chloride were determined based on the S/N ratio. The LOD was calculated to be 0.6 ppm and the LOQ was determined to be 1.8 ppm for all three alkyl chlorides. The precision at the LOQ was assessed by determining the RSD% of LOQ precision, which was found to be <10%. These results are summarized in Table 1.

Linearity

Linear regression analysis of the calibration data for alkyl chlorides showed high correlation coefficients (r^2>0.999) and small percentage y-intercepts (<10.0%). The residual plots demonstrated random scatter, indicating that the method is linear over the calibration ranges of the analytes.

Precision

The precision of the method was evaluated through both method precision and intermediate precision studies. The RSD (%) values for all alkyl chlorides were found to be <5%, confirming that the method provides consistent results. The experimental results from the precision study are summarized in Table 1.

Accuracy

Accuracy was assessed by evaluating the percentage recoveries of alkyl chlorides from spiked samples. The recoveries ranged from 91.0% to 114.0%, falling within the acceptance criteria of 80–120%. These results demonstrate that the method is accurate for the determination of alkyl chlorides. The experimental results from the accuracy study are summarized in Table 1. The chromatograms for both the sample and the LOQ level spiked sample solutions are shown in Figs. 4a-c, respectively, demonstrating the method's ability to accurately and precisely analyze the alkyl chlorides at the specified concentration levels.

Robustness

The robustness of the method was tested by deliberately varying the flow rate and the initial oven temperature. These changes had no significant impact on the resolution or peak shape of the alkyl chlorides, although retention times were altered by the variations in flow rate and oven temperature. This indicates that the method is robust under the tested conditions.

Solution stability

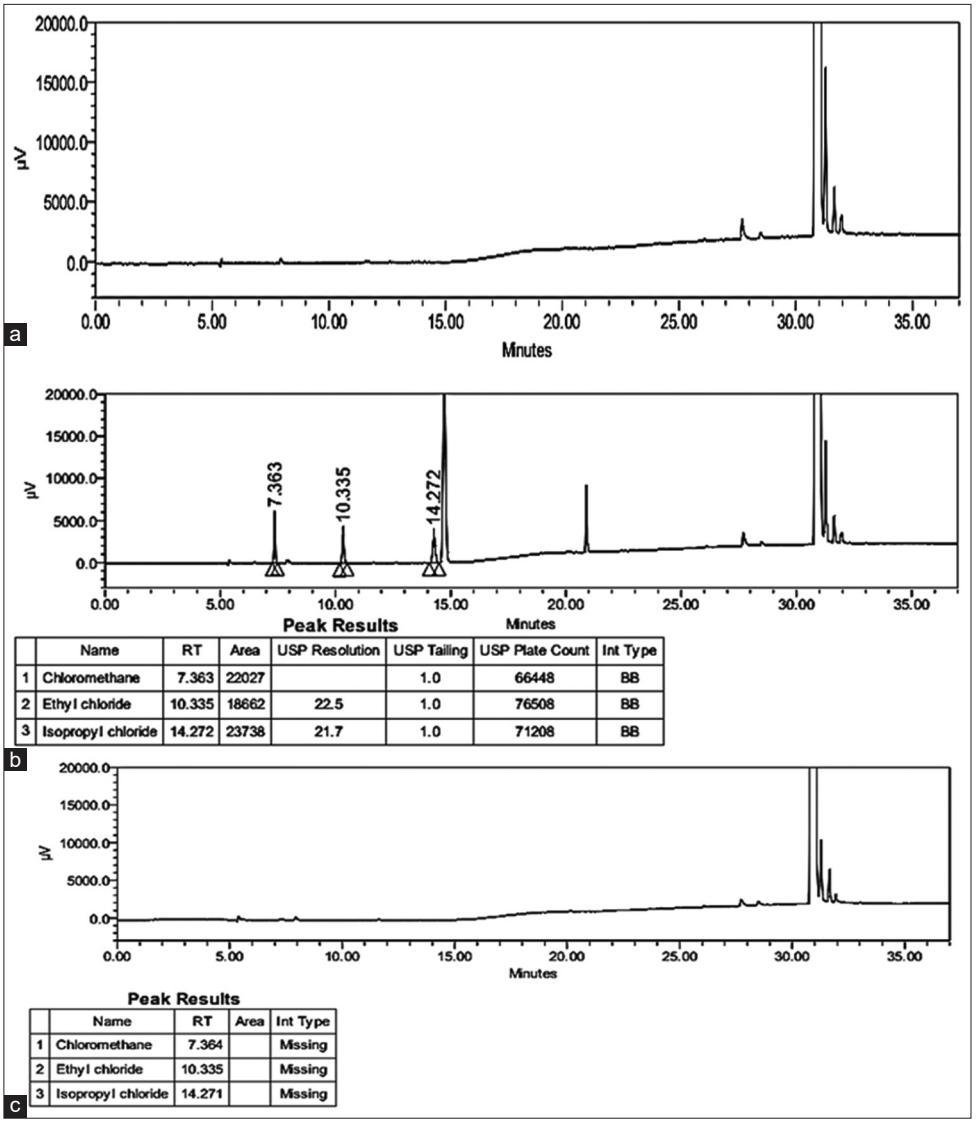
Solution stability studies showed that the standard and spiked sample solutions remained stable for up to 48 h when stored at both ambient laboratory conditions (25±5°C) and refrigerated conditions. The maximum percentage deviation observed was <9.6%, confirming the stability of the solutions over the study period.

The validated method demonstrates excellent specificity across a wide range of solvents used in the synthesis of Trimetazidine Hydrochloride (TMZ), including methanol, ethanol, acetonitrile, isopropyl alcohol, diethyl ether, dichloromethane, tetrahydrofuran, and toluene. The method's robustness allows for the simultaneous determination of these solvents in a single analysis, making it highly efficient for quality control purposes. In addition, the method can be further optimized to assess the presence of degradants, such as solvent impurities or other APIs, that may form during the synthesis or analysis process. Given the high resolution

Table 1: Method validation data summary

Test parameter	Methyl chloride (%)	Ethyl chloride (%)	Isopropyl chloride (%)
System suitability	0.9	1.6	0.7
Specificity	No interference	No interference	No interference
Sensitivity	LOD-0.6 ppm	LOD-0.6 ppm	LOD-0.6 ppm
	LOQ-1.8 ppm	LOQ-1.8 ppm	LOQ-1.8 ppm
	6:1	4:1	4:1
	16:1	11:1	10:1
	2.9	1.7	7.2
Linearity	1.8–27.8 ppm	1.8–27.8 ppm	1.8–27.8 ppm
	y=1184.4×–91.429	y=951.99×+27.434	y=1245.6×+62.171
	0.9998	0.9996	0.9999
	Random scatter	Random scatter	Random scatter
Precision	Average: 92.7	Average: 95.1	Average: 93.8
	RSD: 2.1	RSD: 2.8	RSD: 1.9
Accuracy	LOQ-98.0	LOQ-114.0	LOQ-110.0
	50–99.0	50–98.0	50–97.0
	100–98.0	100–99.0	100–99.0
	150–99.0	150–101.0	150–101.0
Solution stability	Stable for 24 h	Stable for 24 h	Stable for 24 h

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

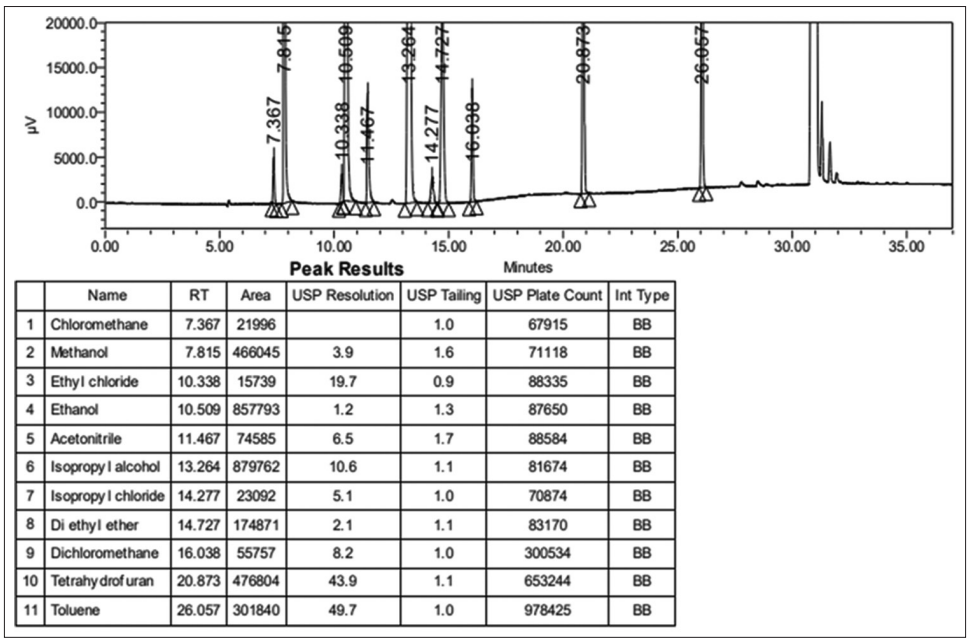


	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	Int Type
1	Chloromethane	7.363	22027		1.0	66448	BB
2	Ethyl chloride	10.335	18662	22.5	1.0	76508	BB
3	Isopropyl chloride	14.272	23738	21.7	1.0	71208	BB

Peak Results

	Name	RT	Area	Int Type
1	Chloromethane	7.364		Missing
2	Ethyl chloride	10.335		Missing
3	Isopropyl chloride	14.271		Missing

Fig. 2: Chromatograms of (a) blank, (b) standard solution, and (c) sample solution



	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	Int Type
1	Chloromethane	7.367	21996		1.0	67915	BB
2	Methanol	7.815	466045	3.9	1.6	71118	BB
3	Ethyl chloride	10.338	15739	19.7	0.9	88335	BB
4	Ethanol	10.509	857793	1.2	1.3	87650	BB
5	Acetonitrile	11.467	74585	6.5	1.7	88584	BB
6	Isopropyl alcohol	13.264	879762	10.6	1.1	81674	BB
7	Isopropyl chloride	14.277	23092	5.1	1.0	70874	BB
8	Di ethyl ether	14.727	174871	2.1	1.1	83170	BB
9	Dichloromethane	16.038	55757	8.2	1.0	300534	BB
10	Tetrahydrofuran	20.873	476804	43.9	1.1	653244	BB
11	Toluene	26.057	301840	49.7	1.0	978425	BB

Fig. 3: Chromatograms of specificity solution containing alkyl halides along with other process solvents

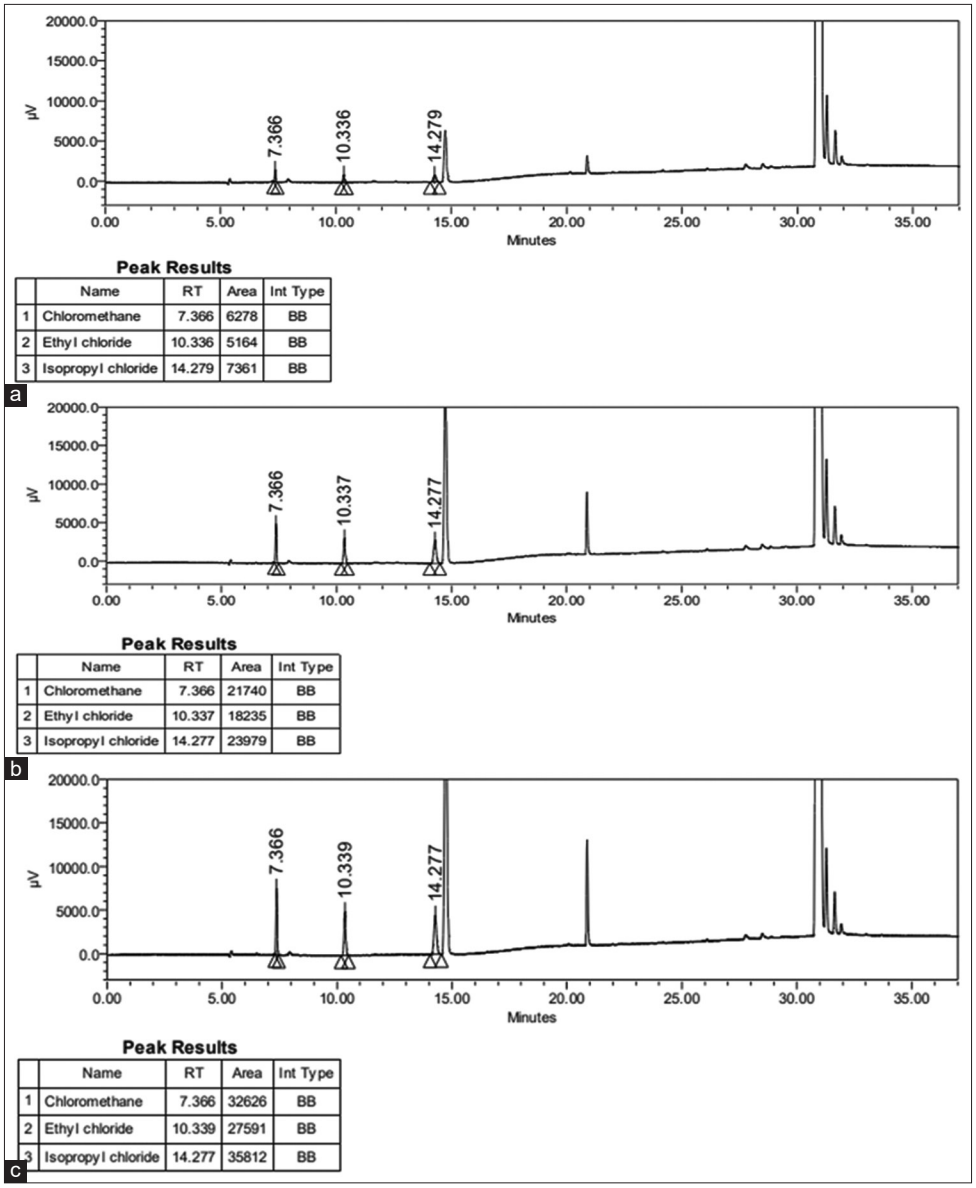


Fig. 4: Chromatograms of spiked sample solution (a) limit of quantification level, (b) 100% level, and (c) 150% level

achieved for all organic solvents, this method holds promise for adapting to other APIs. By establishing the recovery rates for different sample matrices, the method can be tailored for the detection and quantification of various impurities, ensuring the safety and quality of pharmaceutical products. A representative chromatogram is shown in Fig. 3, illustrating the method's ability to clearly resolve all the analytes of interest.

DISCUSSION

The objective of this work was to develop and validate a sensitive, precise, and robust GC-HS method for the quantification of alkyl chloride impurities in pharmaceutical formulations, particularly those containing trimetazidine (TMZ). The method aimed to address challenges posed by matrix interference and the need for trace-level detection of impurities in the presence of high concentrations of TMZ.

The method development and optimization phase were critical for ensuring accurate quantification of alkyl chlorides, particularly in the presence of high concentrations of trimetazidine (TMZ). Initial trials involving different stationary phases, including DB-624, DB-1701, DB-Wax, DB-5, and DB-1, highlighted that DB-1 (100% Dimethylpolysiloxane) provided the best resolution for alkyl chlorides. However, matrix interference from TMZ compromised the peak shape and quantification accuracy. This issue was mitigated using a static HS sampler, which effectively avoided matrix effects, enhancing the sensitivity and resolution of the alkyl chlorides.

Optimizing parameters such as the HS oven temperature, sample line temperature, and flow rate was essential for achieving reliable results. The finalized chromatographic conditions, including a flow rate of 10.2 psi, a split ratio of 1:10, and an oven temperature program ramping from 40°C to 250°C, were selected based on their ability to achieve optimal separation of alkyl chlorides while minimizing matrix interference. These conditions were further validated through a series of performance evaluations following ICH guidelines.

The validation results not only demonstrated the robustness of the method but also highlighted its resilience to experimental variations. Despite changes in the flow rate and oven temperature, the method maintained consistent resolution and peak shape for the target alkyl chlorides, underscoring its stability and reliability under different operating conditions. This robustness is critical for ensuring the method's applicability across varying analytical environments and for routine quality control applications.

The specificity of the method was confirmed by the absence of any interference from matrix components, ensuring that the target alkyl chlorides were accurately quantified without signal overlap from other compounds present in the sample. This was further validated by the linearity of the calibration curves, which showed excellent correlation coefficients (r^2>0.999), demonstrating the method's capability to produce precise and reproducible results over a broad concentration range of 1.8–27.8 ppm. This high degree of linearity suggests that the method is well-suited for quantitative analysis across the typical concentration ranges encountered in pharmaceutical formulations.

In addition, the LOD and LOQ were determined to be 0.6 ppm and 1.8 ppm, respectively. These low detection and quantification limits are indicative of the method's exceptional sensitivity, allowing for the identification and quantification of trace levels of alkyl chlorides in complex samples, such as TMZ formulations. The method's sensitivity ensures its effectiveness in detecting potential contaminants or impurities, which is particularly important for ensuring the purity and safety of the final pharmaceutical product.

Together, these validation results demonstrate the method's suitability for use in routine quality control and regulatory applications, where accurate and sensitive detection of alkyl chlorides is critical for ensuring the safety and efficacy of pharmaceutical products. Moreover, the method's high specificity, robustness, and sensitivity position it as a reliable analytical tool for the analysis of trace level impurities, offering significant advantages in terms of both efficiency and accuracy.

The precision studies, which encompassed both method and intermediate precision, demonstrated exceptional repeatability of the method, with RSD consistently below 5%. This level of precision is well within the acceptable range for analytical methods, indicating that the method can reliably produce consistent results under both intra- and inter-day conditions. Such repeatability is crucial for ensuring the robustness and reliability of the method in routine applications, where consistent results are necessary for both quality control and regulatory compliance.

The accuracy of the method was further confirmed through recovery studies, which revealed recovery rates ranging from 91.0% to 114.0%, all falling within the acceptable recovery range of 80–120%. This range is widely accepted in analytical chemistry as an indicator of reliable and accurate quantification, suggesting that the method is effective at recovering alkyl chlorides from complex matrices without significant loss or contamination. The successful recovery studies reinforce the method's suitability for the quantification of trace alkyl chlorides in pharmaceutical samples, even at low concentrations.

Furthermore, stability studies were performed to assess the method's ability to maintain accuracy over time. The results showed that sample solutions remained stable for up to 48 h, with only minimal deviations in concentration. This finding is particularly important for practical applications, as it demonstrates that the method can be used for analysis without the need for immediate processing of samples, thus offering flexibility in sample handling and analysis. The minimal concentration deviation observed during the stability tests highlights the method's robustness over extended periods, reducing the likelihood of sample degradation or changes in impurity profiles, which is vital for the reliability of analytical results.

Collectively, the precision, accuracy, and stability data presented here demonstrate that the validated method is not only reliable and repeatable but also highly accurate and stable under varying conditions. These results ensure the method's suitability for routine analysis, providing a solid foundation for its use in regulatory and quality control settings. Furthermore, the robustness of the method over extended timeframes enhances its practicality for large-scale applications, ensuring the continued reliability of results across multiple batches and sample types.

The developed GC-HS method exhibits key attributes of robustness, sensitivity, precision, and accuracy, positioning it as an ideal analytical tool for the quantification of alkyl chlorides in pharmaceutical formulations containing TMZ (Temozolomide). Its robustness ensures reliable performance across a range of operating conditions, while its high sensitivity allows for the detection of alkyl chloride impurities at trace levels, which is essential for maintaining the integrity of pharmaceutical formulations. The method's precision, with low RSD, guarantees repeatable results under both intra- and inter-day conditions, further emphasizing its reliability for routine analysis. In addition, the method's accuracy, validated through recovery studies, confirms its ability to provide precise quantification, even in the presence of complex matrices. Given these qualities, this GC-HS method serves as a robust analytical approach that is well-suited for quality control and regulatory compliance in pharmaceutical settings. It ensures the accurate determination of alkyl chloride impurities, enabling manufacturers to meet stringent quality standards and regulatory requirements, ultimately supporting the safety and efficacy of pharmaceutical products.

CONCLUSION

The developed GC-HS method for quantifying alkyl chloride impurities in pharmaceutical formulations containing trimetazidine (TMZ) proved to be highly effective, precise, and reliable. The method demonstrated excellent sensitivity, linearity, accuracy, and robustness, with low LOD and LOQ for methyl chloride, ethyl chloride, and isopropyl chloride. The optimization of experimental conditions, such as the use of the DB-1 column and HS sampling, successfully minimized matrix interference and ensured accurate impurity quantification. In addition, the validation studies confirmed the method's suitability for routine use, with consistent results for precision and stability over extended periods. Overall, this work provides a robust analytical tool for ensuring the quality and safety of pharmaceutical products by reliably quantifying trace-level alkyl chloride impurities, meeting regulatory standards for pharmaceutical analysis.

ACKNOWLEDGMENT

The authors express their sincere gratitude to the analytical facility of JNTUH and Sai Life Sciences for granting access to their analytical instruments.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the current work.

DATA AVAILABILITY STATEMENT

The data of the present study are available from the corresponding author on reasonable request.

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.

FUNDING

None.

REFERENCES

1. Yang J, Zhang L, Liu C, Zhang J, Yu S, Yu J, *et al*. Trimetazidine attenuates high-altitude fatigue and cardiorespiratory fitness impairment: A randomized double-blinded placebo-controlled clinical trial. Biomed Pharmacother. 2019;116:109003. doi: 10.1016/j.biopha.2019.109003, PMID 31125823
2. McClellan KJ, Plosker GL. Trimetazidine. A review of its use in stable angina pectoris and other coronary conditions. Drugs. 1999;58(1):143-57. doi: 10.2165/00003495-199958010-00016, PMID 10439934
3. Vitale C, Marazzi G, Pelliccia F, Volterrani M, Cerquetani E, Spoletini I,

et al. Trimetazidine improves exercise performance in patients with peripheral arterial disease. Pharmacol Res. 2011;63(4):278-83. doi: 10.1016/j.phrs.2011.01.003, PMID 21220024

4. Sigmund G, Koch A, Orlovius AK, Guddat S, Thomas A, Schänzer W, *et al*. Doping control analysis of trimetazidine and characterization of major metabolites using mass spectrometric approaches. Drug Test Anal. 2014;6(11-12):1197-205. doi: 10.1002/dta.1680, PMID 24913825
5. Giordani A, Kobel W, Gally HU. Overall impact of the regulatory requirements for genotoxic impurities on the drug development process. Eur J Pharm Sci. 2011;43(1-2):1-15. doi: 10.1016/j.ejps.2011.03.004, PMID 21420491
6. Yang Q, Haney BP, Vaux A, Riley DA, Heidrich L, He P, *et al.* Controlling the genotoxins ethyl chloride and methyl chloride formed during the preparation of amine hydrochloride salts from solution and ethanol and methanol. Org Process Res Dev. 2009;13(4):786-91. doi: 10.1021/op9000737
7. Müller L, Mauthe RJ, Riley CM, Andino MM, Antonis DD, Beels C, *et al*. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regul Toxicol Pharmacol. 2006;44(3):198-211. doi: 10.1016/j.yrtph.2005.12.001, PMID 16412543
8. Snodin DJ. Genotoxic impurities: From structural alerts to qualification. Org Process Res Dev. 2010;14(4):960-76. doi: 10.1021/op100118e
9. European Medicines Agency (EMEA), Committee for Medicinal Products for Human Use (CHMP). Guideline on the Limits of Genotoxic Impurities, CPMP/SWP/5199/02. In: Guideline on Genotox Impurities-Final-SWP Number-Superseded Watermarked Jul 2019 doc; 2006. Available from: https://www.europa.eu
10. USFDA. S2 (R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use. ICH S2 (R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use-Scientific Guideline. In: European Medicines Agency (EMA); 2012. Available from: https://www.europa.eu
11. ICH. M7 (R1): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk; 2017. Available from: https://database.ich.org/sites/default/files/m7_r1_guideline.pdf
12. Sultana S, Nagarajan B. Simultaneous trace-level determination of benzene and 1,2-dichloroethane by GC-HS/GC-MS in several pharmaceutical drug substances. Int J Appl Pharm. 2019;11(1):82-8. doi: 10.22159/ijap.2019v11i1.28983
13. Aparna K, Rachel KV, Rao KM. Quantitative determination of methyl-4-chlorobutyrate, a potential genotoxic impurity, content in moxifloxacin HCl by GC-EI-MS. Int J Appl Pharm. 2024;16(5):234-41. doi: 10.22159/ijap.2024v16i5.51551
14. Hughes RA, Knighton WB, Grimsrud EP. Enhancement of electron-capture detection of methyl bromide in air by iodination. J Chromatogr A. 1999;852(2):535-43. doi: 10.1016/S0021-9673(99)00649-4, PMID 10481990
15. Narapereddy KP, Alladi DS. Development and validation of determination of genotoxic impurity bromoethane in vigabatrin drug substance using head space gas chromatographic method [HS-GC]. Pharmacia. 2023;70(1):203-07. doi: 10.3897/pharmacia.70.e97339
16. Van Wijk AM, Beerman B, Niederländer HA, Siebum AH, De Jong GJ. A new approach for generic screening and quantitation of potential genotoxic alkylation compounds by pre-column derivatization and LC-MS/MS analysis. Anal Bioanal Chem. 2011;400(5):1375-85. doi: 10.1007/s00216-011-4901-y, PMID 21445660
17. Rajesh Varma B, Srinivas Rao B, Kapavarapu MN, Varaprasad Reddy M. Assessment of gas chromatography methodology approach for the trace evaluation of carcinogenic impurity, methyl chloride, in trimetazidine dihydrochloride. Ann Pharm Fr. 2023;18(1):64-73. doi: 10.1016/j.pharma.2022.06.012
18. ICH Q2A(R1). Validation of analytical Procedures: Text and Methodology; 2005. Available from: https://database.ich.org/sites/default/files/q2%28r1%29%20guideline.pdf
19. Patha RP, Dasa K, Bhoomireddy RD, Thumu SR. *In-silico* toxicity assessment and trace level quantification of veratryl chloride, a potential genotoxic impurity in ivabradine hydrochloride using LC-MS/MS. Int J Pharm Sci Drug Res. 2023;15(4):488-93. doi: 10.25004/IJPSDR.2023.150413
20. US FDA. Analytical Procedures and Methods Validation for Drugs and Biologics; 2015. Available from: https://www.fda.gov/files/drugs/published/analytical-procedures-and-methods-validation-for-drugs-and-biologics.pdf