

Article

Development and Validation of a Solvent-Free Headspace GC-MS Method for the Screening of Benzyl Chloride in Pharmaceutical Products

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Abstract: This study presents a solvent-free headspace gas chromatography–mass spectrometry (SF-HS-GC/MS) method for robustly screening benzyl chloride, a mutagenic carcinogen, impurities in active pharmaceutical ingredients (APIs) and drug products. The SF-HS-GC/MS method simplifies analysis by eliminating solvent use, reducing matrix interference. Optimized headspace parameters include incubation temperature, time, and sample amount. Validation, aligned with Q2(R1) ICH guidelines and ICH M7 recommendations, covers selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, system suitability, and robustness. Employing a DB-5MS column (30 m × 0.25 mm, 0.25 μm) with solvent-free split injection, the method’s calibration curve (0.05–5 μg/g) exhibits a strong correlation (>0.9998). The LOQ was 0.1 μg/g, with precision (%CV) consistently <5% and accuracy within 95–105%. Furthermore, an investigation confirmed the absence of artefactual benzyl chloride formation in drug products under headspace conditions. The developed SF-HS-GC/MS method successfully screened benzyl chloride in cinnarizine drug substances and products.

Keywords: benzyl chloride; cinnarizine; ICH M7; impurity; solvent-free HS-GC/MS



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1. Introduction

In recent years, the presence of a carcinogenic impurity in pharmaceuticals, exemplified by N-nitrosodimethylamine, has raised significant public concerns [1–17]. Carcinogenic impurities can potentially form during the production and storage of active pharmaceutical ingredients (APIs) and drug products [18]. To address the potential risk of carcinogenic impurities, international efforts have been made, including the implementation of the ICH M7(R1) guideline by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use [19].

Benzyl halides are commonly used compound in the synthesis of APIs, particularly as arylation reagents [20,21]. Several APIs that may undergo arylation using benzyl chloride in the synthesis steps have been identified, including cinnarizine, which serves as the primary API for method validation in this study [22]. However, it is important to note that benzyl chloride is not detected in cinnarizine itself. It is also reported that benzyl chloride can form as an intermediate during the synthetic route [23]. For instance, benzyl alcohol

has the potential to undergo conversion to the corresponding chloride, which may persist in the continuous multistage synthesis of APIs.

Benzyl chloride, widely used as a reagent, is a controlled impurity specified in ICH M7(R1) and classified Class 1, “Known mutagenic carcinogens” [24]. According to the ICH M7(R1) guidelines, the acceptable intake (AI) of benzyl chloride as a potential impurity in APIs is 41 µg/day, interpolated from TD50 (tumorigenic dose, the estimated carcinogenic potency) [19]. However, from a more conservative perspective, employing more stringent criteria, such as the Threshold of Toxicological Concern (TTC), is desirable for benzyl chloride. The TTC is generally based on chronic or lifetime exposure and applies to compounds with mutagenicity but lacking carcinogenic data (Class 2 or 3). For a 60 kg body weight, the TTC for benzyl chloride is set at 1.5 µg/day [19]. Notably, the TTC criterion of 1.5 µg/day is significantly lower than the AI of 41 µg/day.

Benzyl chloride can be analyzed using various analytical techniques, including high-performance liquid chromatography (HPLC) [25], gas chromatography (GC) [26,27], and gas chromatography–mass spectrometry (GC-MS) [28–30]. For example, in the analysis of benzyl chloride in environmental samples, HPLC with a reversed-phase C18 column (150 × 3.2 mm, 5 µm) coupled with a UV detector has been employed. This method achieved a limit of detection (LOD) of 90 ppt through intensive sample preconcentration in [25]. GC with flame ionization detection (GC-FID) using a nonpolar DB-1701 or DB-1 column was utilized to analyze benzyl chloride in tear gas compounds [26]. Benzyl chloride in indoor volatile organic compounds (VOCs) in childcare facilities was determined using GC-MS with a nonpolar DB-5MS (30 m × 0.25 mm, 1 µm), achieving an LOD of 0.001 µg/m³ and a limit of quantification (LOQ) of 0.002 µg/m³ [30].

Headspace gas chromatography–mass spectrometry (HS-GC/MS) has also been employed for the analysis of benzyl chloride in processed food and products [31]. In this method, the LOD for benzyl chloride ranged from 0.04 to 0.17 mg/kg while the LOQ varied from 0.13 to 0.52 mg/kg. The analysis utilized a nonpolar HP-1 column (0.32 mm × 30 m, 0.25 µm). It is worth noting that this approach incorporated headspace sampling with a solvent-based method during the sample introduction step, which added complexity to the analytic process. To enhance the sensitivity of these foundational analytical techniques, headspace solid phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC/MS) have also been employed for benzyl chloride analysis [32,33].

Most analytical studies utilizing headspace equipment have employed solvent-based analysis methods [31–35]. In these studies, the accuracy of benzyl chloride analysis is significantly influenced by the sample matrix. Conventional HS-GC/MS methods typically involve the use of solvents for sample preparation, which can introduce matrix effects. Additionally, the low boiling point of benzyl chloride (179 °C) renders it susceptible to instability during the sample preparation step, potentially leading to sample loss and affecting the concentration of the sample during the workup process [16]. These challenges can affect the accuracy and sensitivity of the analysis.

On the other hand, solvent-free HS-GC/MS (SF-HS-GC/MS) enables the direct analysis of volatile compounds from a sample without the need for sample preparation [36]. This method enhances sample integrity by minimizing sample handling processes. It reduces the risk of sample degradation and minimizes matrix interference, thereby enabling sensitive detection of target compounds [16].

In this study, we developed a novel SF-HS-GC/MS method using selected ion monitoring mode (SIM) to screen for the presence of benzyl chloride in APIs and drug products, following the Q2(R1) ICH guidelines [37]. It is important to note that this method is specifically designed for the screening of benzyl chloride in APIs and drug products rather than for accurate quantitative analysis. This is due to the rare potential for artificial formation of benzyl chloride during the analytical process. The developed method will undergo validation for selectivity, linearity, LOD, LOQ, precision, accuracy, system suitability, and robustness. The method proved effective in accurately determining trace levels of benzyl chloride in various APIs and drug products. The study examined the drug products of

cinnarizine, entecavir, cetirizine, levocetirizine, and meclizine (refer to Figure 1) as they may potentially contain benzyl chloride as an impurity [22,23]. Cinnarizine, an antihistamine that inhibits smooth muscle cell contraction in the vasculature by blocking L- and T-type voltage-gated calcium channels, was selected as the primary API for method validation. Additionally, to ensure the absence of artefactual formation of benzyl chloride, we conducted an additional study by modifying the headspace parameters for the API and drug products.

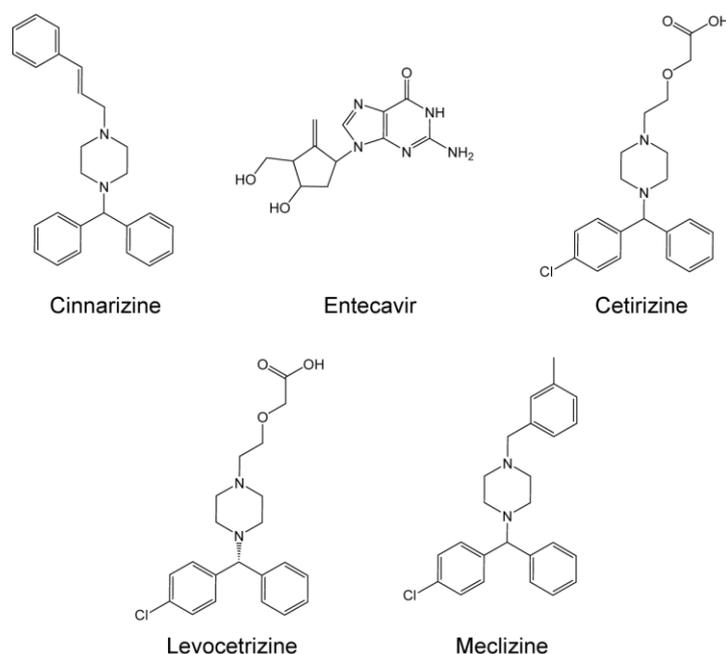


Figure 1. Structures of APIs.

2. Materials and Methods

2.1. Materials and Reagents

Benzyl chloride (purity: 99%) and benzyl chloride- d_7 (purity: 98%) as the internal standard were purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was also obtained from Sigma Aldrich. HPLC-grade methanol was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Cinnarizine, the main API, was obtained from Tokyo Chemical Industry (TCI, Chuo-Ku, Tokyo, Japan), and the drug products used in this study were acquired through Korean regulatory agencies. Headspace vials (20 mm/20 mL) and caps (Headspace anal. crimp, PTFE/silicone septum) were obtained from Agilent Technologies (Santa Clara, CA, USA).

2.2. Preparation of Standard Solutions

Stock solutions of benzyl chloride and the internal standard benzyl chloride- d_7 were prepared in methanol at a concentration of 1000 $\mu\text{g}/\text{mL}$. Working standard solutions were then prepared by diluting the stock solutions to obtain concentrations of 0.25, 0.5, 2.5, 5, 7.5, 10, 12.5, and 25 $\mu\text{g}/\text{mL}$, using an appropriate volume of methanol. A 10 μL aliquot of the working standard solution was directly added to 50 mg of the drug product, resulting in final benzyl chloride sample concentrations of 0.05, 0.1, 0.5, 1, 1.5, 2, 2.5, and 5 $\mu\text{g}/\text{g}$. Furthermore, a working standard solution of the internal standard was prepared by diluting the stock solution to a concentration of 5 $\mu\text{g}/\text{mL}$. Subsequently, 10 μL of this internal standard working solution was added to 50 mg of the drug product, resulting in a final internal standard concentration of 1 $\mu\text{g}/\text{g}$.

2.3. Sample Preparation

Six tablets of drug product samples were individually ground into a powder for the experiment. The SF-GC/MS method was conducted following the Q2(R1) ICH guidelines [37]. For the cinnarizine solid powder sample, a 50 mg portion was directly placed into the headspace vial without the addition of any solvent. The vial was then placed into the autosampler, incubated at 110 °C for 10 min, and subsequently analyzed using GC-MS.

2.4. HS-GC/MS Conditions

GC-MS analysis was performed on an Agilent 6890N gas chromatograph equipped with an Agilent 5973 mass spectrometer, utilizing a DB-5MS column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). Optimization of headspace conditions was conducted by testing the incubation temperature, incubation time (equilibration time), and sample amount. Benzyl chloride spiked in the API, i.e., cinnarizine, was analyzed using a headspace method with an equilibration time of 10 min at 110 °C. To prevent carryover from the previous sample, the loop temperature was set at 125 °C, and the transfer-line temperature was set higher than the loop temperature at 140 °C. The headspace parameters were set with a loop fill time of 0.5 min, loop equilibrium time of 0.1 min, pressurizing time of 1 min, and injection time of 1 min. The inlet temperature was set at 250 °C. The headspace sample was injected in a split mode of 20:1 with an injection volume of 1.0 µL. The column oven temperature was set at 50 °C, ramped up to 120 °C at a rate of 15 °C/min, and then increased to 300 °C at a rate of 30 °C/min and held for 5 min. For the MS parameters, the ion source temperature was 250 °C, and the quad temperature was set at 150 °C. The electron ionization (EI) energy was set at 70 eV. The acquisition type was selected as SIM mode, with m/z 91 selected for benzyl chloride and m/z 98 for benzyl chloride- d_7 . The dwell time of each ion was set at 50 ms.

2.5. Method Validation

The validated method parameters include selectivity, linearity, LOD, LOQ, precision, accuracy, system suitability, and robustness. Linearity was evaluated by preparing calibration standards through spiking stock solutions into the API, i.e., cinnarizine, at eight concentration points (0.05, 0.1, 0.5, 1, 1.5, 2, 2.5, and 5 µg/g) in triplicate. The calibration curve was derived by performing linear regression analysis on the peak ratio of benzyl chloride to its deuterium-labeled internal standard in relation to its concentration.

Accuracy was assessed using inter-day and intra-day measurements in triplicate for three consecutive days. Quantification of the method was performed by introducing the established quantity of the benzyl chloride working standard into the API, i.e., cinnarizine, and the drug product. Standards of 0.1, 1, and 4 µg/g benzyl chloride were spiked, which corresponded to low, medium, and high concentrations, respectively. Precision was also evaluated by calculating the inter-day and intra-day deviations for cinnarizine and the drug product.

3. Results and Discussion

3.1. Headspace Parameter Optimization

To compare the efficiency of three GC/MS-based analysis methods for screening benzyl chloride in the API, i.e., cinnarizine, we employed GC-MS, conventional HS-GC/MS, and SF-HS-GC/MS methods in SIM mode. The resulting chromatograms (Figure 2) were obtained by adding a benzyl chloride working standard solution to the API, resulting in a final concentration of 5 µg/g. The peak for benzyl chloride appeared at 4.54 min. The chromatogram obtained using the SF-HS-GC/MS method (a) displayed significantly higher sensitivity than those obtained with GC-MS (b) and the conventional solvent-based HS-GC/MS method (c). The SF-HS-GC/MS method provides significantly enhanced sensitivity for screening benzyl chloride in the API compared with other solvent-based approaches.

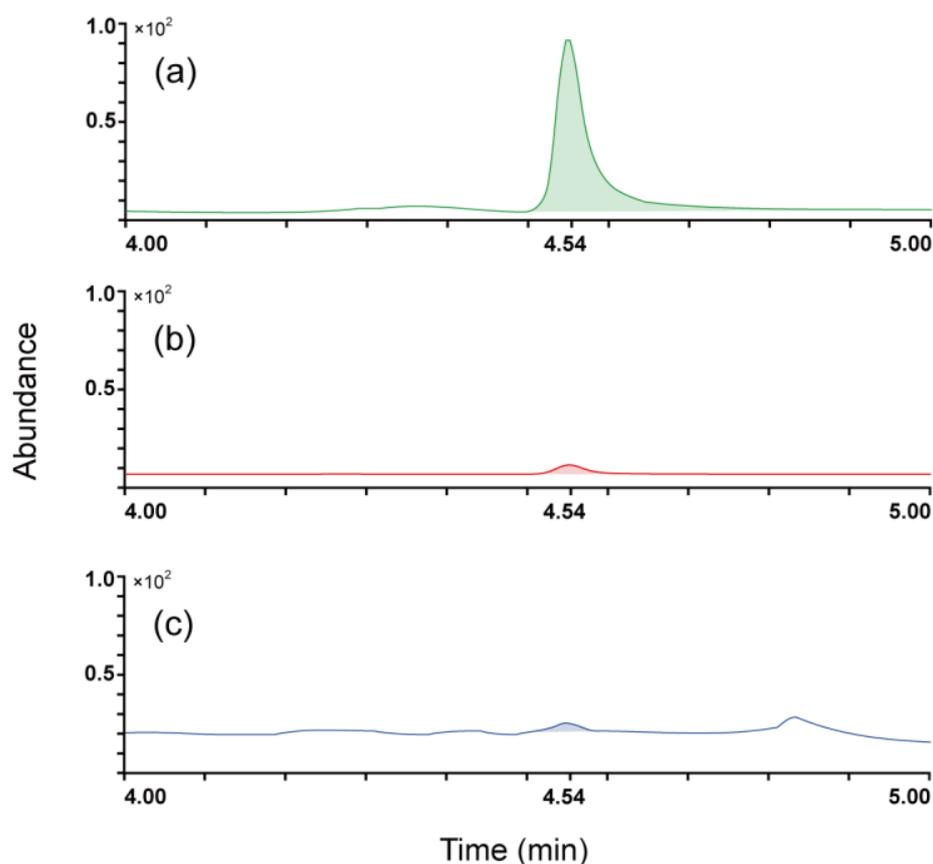


Figure 2. Ion chromatograms of benzyl chloride for spiked drugs (5 µg/g level) by (a) solvent-free HS-GC/MS, (b) GC/MS, and (c) conventional HS-GC/MS.

To effectively screen benzyl chloride in the API and drug products using the SF-HS-GC/MS method, several experimental parameters were optimized. Included parameters were incubation temperature, incubation time, loop temperature, transfer-line temperature, loop fill time, loop equilibration time, pressurizing time, injection time, and sample amount. Among these parameters, it was found that three parameters—namely, incubation temperature, incubation time, and sample amount—had the most significant impact on benzyl chloride screening.

First, the incubation temperature was varied from 100 °C to 160 °C at intervals of 10 °C for spiked headspace samples. In the optimization experiments, benzyl chloride was added to the API at a concentration of 0.3 µg/g, also with the internal standard benzyl chloride- d_7 . The average chromatographic peak area decreased rapidly from 100 °C to 130 °C and flattened above 130 °C (Figure 3a, left panel). The peak area ratios between the peaks of benzyl chloride and internal standard are more or less similar from 100 °C to 150 °C but rapidly increased at 160 °C (Figure 3a, right panel). The relative standard deviation (RSD) of the average chromatographic peak area ratio was the smallest at 110 °C, enabling sensitive detection of benzyl chloride with great reproducibility.

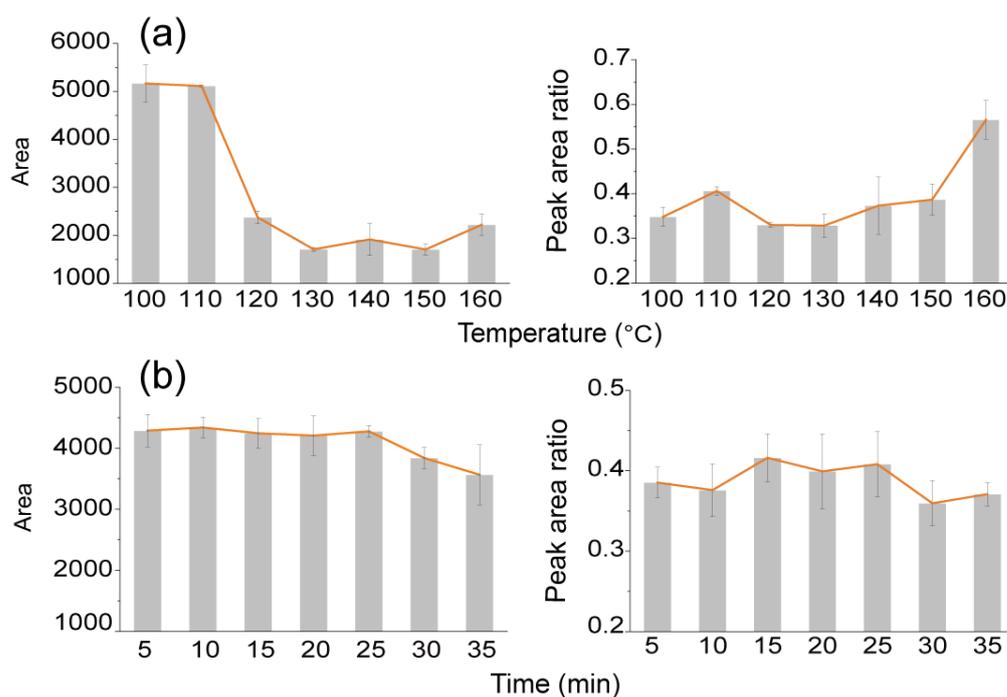


Figure 3. Optimization of solvent-free HS conditions according to the variations in (a) incubation temperature and (b) incubation time.

Second, the incubation time was varied from 5 to 35 min at intervals of 5 min. The peak area slowly decreased with the incubation time while the RSD of the chromatographic peak area increased as the incubation temperature increased (Figure 3b, left panel). The peak area ratios were similar for incubation times from 5 min to 35 min (Figure 3b, right panel). Based on these findings, an incubation time of 10 min was selected for subsequent experiments.

Lastly, the amount of drug sample was varied from 20 to 100 mg, with an incubation temperature of 110 °C and an incubation time of 10 min. A sample amount of 50 mg showed the lowest RSD in both the peak area and the peak area ratio. Therefore, the solvent-free headspace conditions were optimized at an incubation temperature of 110 °C, incubation time of 10 min, and sample amount of 50 mg, allowing for sensitive detection with high reproducibility. Furthermore, to avoid carryover from previous samples, the temperature of the loop was set 15 °C higher than the incubation temperature. The transfer-line temperature was also set 15 °C higher than the loop temperature.

3.2. Method Validation

3.2.1. Calibration Curve and Linearity

To assess the linearity of the SF-HS-GC/MS measurements, a calibration curve was generated by analyzing benzyl chloride concentrations ranging from 0.05 to 5 µg/g, along with a fixed internal standard concentration of 1 µg/g. To achieve this, 50 mg of the API, spiked with a benzyl chloride working standard solution containing various concentrations of benzyl chloride and a consistent amount of the internal standard, was directly added to the headspace vial. The resulting peak ratio of benzyl chloride to its deuterium-labeled internal standard was used to plot the calibration curve, which was then analyzed using linear regression analysis. The calibration equation and linear correlation coefficient of benzyl chloride measurements can be found in Table 1. The obtained R^2 value of 0.9997 indicates excellent linearity of benzyl chloride within the specified concentration range.

Table 1. Method validation results for the analysis of benzyl chloride using SF-HS-GC/MS-SIM method.

Contents	Linear Range (µg/g)	Calibration Equation	Correlation Coefficient (R ²)	Con. (µg/g)	Accuracy and Precision			
					Intra-Day (n = 3)		Inter-Day (n = 3)	
					Accuracy (%) ^a	RSD (%) ^b	Accuracy (%) ^a	RSD (%) ^b
API	0.5–25	y = 1.25x – 0.0419	0.9997	0.1	101.4	4.52	99.8	3.75
				1	102.9	2.54	103.8	3.77
				4	100.4	1.84	100.0	3.57
Drug product	0.5–25			0.1	98.2	1.45	100.3	1.61
				1	98.9	3.28	100.3	2.86
				4	95.6	2.27	97.9	1.71

^a Accuracy (%) = (the mean concentration of measured standard solution/the concentration of spiked sample) × 100; ^b RSD (%) = (standard deviation/mean) × 100.

3.2.2. Accuracy and Precision

To assess the accuracy and precision of the SF-HS-GC/MS method, intra-day and inter-day experiments were conducted, respectively. Benzyl chloride was spiked into both the API, i.e., cinnarizine, and the drug product (50 mg), resulting in final sample concentrations of 0.1, 1, and 4 µg/g. The analysis results are presented in Table 1.

For intra-day accuracy, triplicate tests were performed, and the RSD was consistently below 5% for all concentrations. Similarly, for inter-day accuracy, the RSD remained below 5% for all concentrations, with lower variability observed in the drug product. The intra-day accuracy range was found to be between 100.4% and 101.4% for the API and between 95.6% and 98.2% for the drug product. Meanwhile, the inter-day accuracy range was between 99.8% and 103.8% for the API and 97.9% and 100.3% for the drug product.

Regarding precision, the intra-day RSD ranged from 1.84% to 4.52% for the API and 1.45% to 3.28% for the drug product. The inter-day RSD ranged from 3.57% to 3.75% for the API and 1.61% to 2.86% for the drug product. The RSD value below 5% and the accuracy range of 95–105% demonstrate the reproducibility and precision of the developed SF-HS-GC/MS method for the screening of benzyl chloride in APIs and drug products. Furthermore, it is noteworthy that the precision and accuracy achieved during both intra-day and inter-day experiments align with the stringent general criteria stipulated by the

Korean Ministry of Food and Drug Safety. The RSD values remained well below the recommended threshold of 20%, indicating a commendable level of precision. Additionally, the accuracy results fell within the predefined range of 80% to 120%, serving to underscore the robustness and reliability of our findings.

It is also important to highlight that benzyl chloride analysis in pharmaceuticals can be significantly influenced by solvent effects [35]. However, the SF-HS-GC/MS method eliminates this solvent effect, as evidenced by the experimental results presented in Table 1.

3.2.3. LOD and LOQ

The LOD and LOQ are crucial parameters that assess the sensitivity of an analytical method. The LOD represents the lowest concentration of an analyte in a sample matrix that can be reliably distinguished from background noise or other interfering signals based on the signal-to-noise (S/N) ratio. In our study, the LOD was determined to be 0.03 µg/g in a sample matrix of 50 mg, using the criteria of S/N ≥ 3. On the other hand, the LOQ refers to the lowest concentration of an analyte in a sample matrix (50 mg) that can be accurately and precisely quantified. This measure requires both detection and quantification of the

analyte, also relying on the S/N ratio. In our study, we established the LOQ at 0.1 µg/g with a criterion of $S/N \geq 10$. Remarkably, the determined LOQ is significantly lower than the TTC for benzyl chloride, which stands at 1.5 µg/day. It is crucial to emphasize that both the LOD and LOQ values comfortably reside beneath the TTC threshold, thereby reaffirming the method's robust capacity to accurately discern even the minutest traces of benzyl chloride, if present, well below the defined TTC threshold.

3.2.4. System Suitability and Robustness

System suitability was evaluated by measuring the retention times of benzyl chloride and its deuterium-labeled internal standard. It also included the peak areas of benzyl chloride and the internal standard, the peak area ratios, and their corresponding RSD values. As shown in Table 2, the obtained RSD values were all below 5%, affirming the suitability of this method for the investigated system.

Table 2. System suitability results for benzyl chloride analysis.

Sample	RT (min)	Peak Area	IS RT	IS Peak Area	Peak Area Ratio
1	4.56	865	4.52	13365	0.0647
2	4.56	823	4.52	12706	0.0648
3	4.56	794	4.52	12050	0.0659
4	4.57	800	4.52	12134	0.0659
5	4.56	780	4.52	11862	0.0658
6	4.57	843	4.52	12655	0.0666
RSD (%)	0.001	3.95	0.0001	4.4671	1.1205

To demonstrate the robustness of the method, several modifications were implemented. Firstly, the flow rate was adjusted by ± 0.1 mL/min, representing a 10% change from the initial rate of 1.0 mL/min. Secondly, the initial temperature was modified by ± 5 °C from the original starting point of 50 °C. The results of these alterations are summarized in Table 3, where all %CV (coefficient of variation) values were found to be below 5%. These findings substantiate the robustness and reliability of the method.

Table 3. Robustness results of the benzyl chloride analysis.

Contents	Value	Benzyl Chloride (1 µg/g)	
		Accuracy (%)	Rate (%)
Flow rate (mL/min)	0.9	94.3	3.19
	1.1	100.5	3.22
Initial temp. (°C)	45	103.0	4.70
	55	104.7	5.84

3.2.5. Inter-Laboratory Cross-Validation

To evaluate the accuracy and precision of the method, an inter-laboratory cross-validation was conducted in collaboration with Kyung Hee University. Six replicates of the API (specifically, cinnarizine) and the drug product at a concentration of 4 µg/g were tested. The precision of the method, represented by the RSD, was determined to be 6% or less, as presented in Table 4. Furthermore, the maximum range of accuracy was found to be below 105%. These outcomes, derived from the inter-laboratory cross-validation, provide additional evidence supporting the effectiveness of this method for screening benzyl chloride in the drug product. To summarize, as partially shown in Figure 3, optimization was carried out to determine the optimal experimental conditions. This allows for low RSD and, ultimately, eliminates the possibility of artefactual formation of benzyl chloride (see below).

Table 4. Inter-laboratory cross-validation results for benzyl chloride.

Laboratory	Compound	Concentration (µg/g)	Average (µg/g)	RSD (%)	Accuracy (%)
Laboratory 1*	API (STD)	4	3.94	4.68	98.6
	Drug product		3.97	5.96	99.4
Laboratory 2**	API (STD)	4	4.18	2.33	104.5
	Drug product		3.99	5.45	99.6

Laboratory 1*: Sogang University, Laboratory 2**: Kyung Hee University.

3.2.6. Comparison with Other Studies

When comparing our research results with other methods such as HPLC, GC, GC-MS, and SPME-GC/MS, the LOD of the HPLC method for analyzing benzyl chloride in environmental samples was determined at 90 ppt, indicating higher sensitivity compared with our method [25]. While this method demonstrated impressive sensitivity, their drawback lies in the lengthy preconcentration steps, leading to increased analysis time. In contrast, our SF-HS-GC/MS method offers the advantage of rapid and convenient analysis without the need for preconcentration.

Furthermore, the GC/MS method exhibited significant sensitivity, with LOD and LOQ values of 0.020 ppbv and 0.100 ppbv, respectively, for the analysis of benzyl chloride in VOCs [28]. Similarly, other studies investigating benzyl chloride in VOCs reported LOD values of 1 ppb and LOQ values of 3.1 ppb [29]. While these studies demonstrated heightened sensitivity, our solvent-free approach minimizes matrix effects, enhancing accuracy, precision, and analysis speed. Upon reviewing the actual experimental results presented in Figure 2, it becomes clear that our HS-SF-GC/MS method for pharmaceutical analysis outperforms the GC/MS method in terms of accuracy, speed, and ease of use.

Moreover, HS-SPME-GC/MS has proven useful for analyzing benzyl chloride and related compounds in water, with an LOD of 20 ng/L [32]. Additionally, an HS-SPME-GC/FID method was developed to analyze benzyl chloride as an impurity in pharmaceutical processes, yielding LOD and LOQ values of 0.3 ng/mL and 0.9 ng/mL, respectively [35]. However, this study reported lower accuracy at 74.1%. In comparison, accuracy fell within the acceptable range of 95–105%, demonstrating even higher levels of accuracy in our study.

Under these conditions, our study confirms that despite exhibiting relatively lower sensitivity compared with highly sensitive methods, our method satisfies the criteria of being sufficiently sensitive, as indicated by LOD and LOQ values lower than the established TTC for benzyl chloride. These outcomes underscore the dependable sensitivity of our research in benzyl chloride analysis. Moreover, our approach focuses on minimizing preconcentration steps and emphasizes the advantages of convenience and rapid analysis.

3.3. Possibility of Artefactual Formation of Benzyl Chloride during SF-HS-GC/MS

The SF-HS-GC/MS method utilized in this study for benzyl chloride detection is widely recognized for its sensitivity and accuracy. However, it is crucial to acknowledge the possibility of artefactual formation of benzyl chloride under the specific experimental conditions employed. To address this concern, three key optimization factors—namely, incubation temperature, incubation time, and sample amount—were meticulously adjusted to monitor any potential formation of benzyl chloride.

To investigate whether benzyl chloride impurities were generated or if there was any artefactual production of benzyl chloride during the headspace process or GC analysis, experiments were conducted without the addition of benzyl chloride to the drug product. The obtained results clearly demonstrate the absence of benzyl chloride formation under the optimized parameters mentioned.

However, in the event that benzyl chloride is detected, further confirmation may be required using a more stable liquid chromatography–mass spectrometry (LC/MS) method for subsequent analysis. This additional step ensures a comprehensive examination of

any potential artefacts or unexpected sources of benzyl chloride, thereby enhancing the reliability and validity of the analytical findings.

3.4. Method Applications

The SF-HS-GC/MS method developed in this study was applied to screen for the presence of benzyl chloride in various drug products (50 mg). The drug products under examination included entecavir, cetirizine, levocetirizine, and meclizine, as well as the drug product cinnarizine (refer to the structures in Figure 1). The method utilized the peak at m/z 91 to monitor the presence of benzyl chloride, with m/z 98 serving as the internal standard. The results showed that none of the screened drug products contained detectable or quantifiable levels of benzyl chloride. To assess the method's sensitivity in detecting benzyl chloride, a benzyl chloride working standard solution of 0.5 $\mu\text{g}/\text{mL}$ was added to several drug products, resulting in a concentration of 0.1 $\mu\text{g}/\text{g}$. The resulting chromatograms are presented in the right panels of Figure 4, clearly showing the presence of the benzyl chloride peak. A comparison of chromatograms with and without benzyl chloride demonstrated the effectiveness of the SF-HS-GC/MS method in distinguishing the absence and presence of benzyl chloride in various drug products. This confirmed the viability of this solvent-free technique as a means of detecting and screening benzyl chloride in drug products.

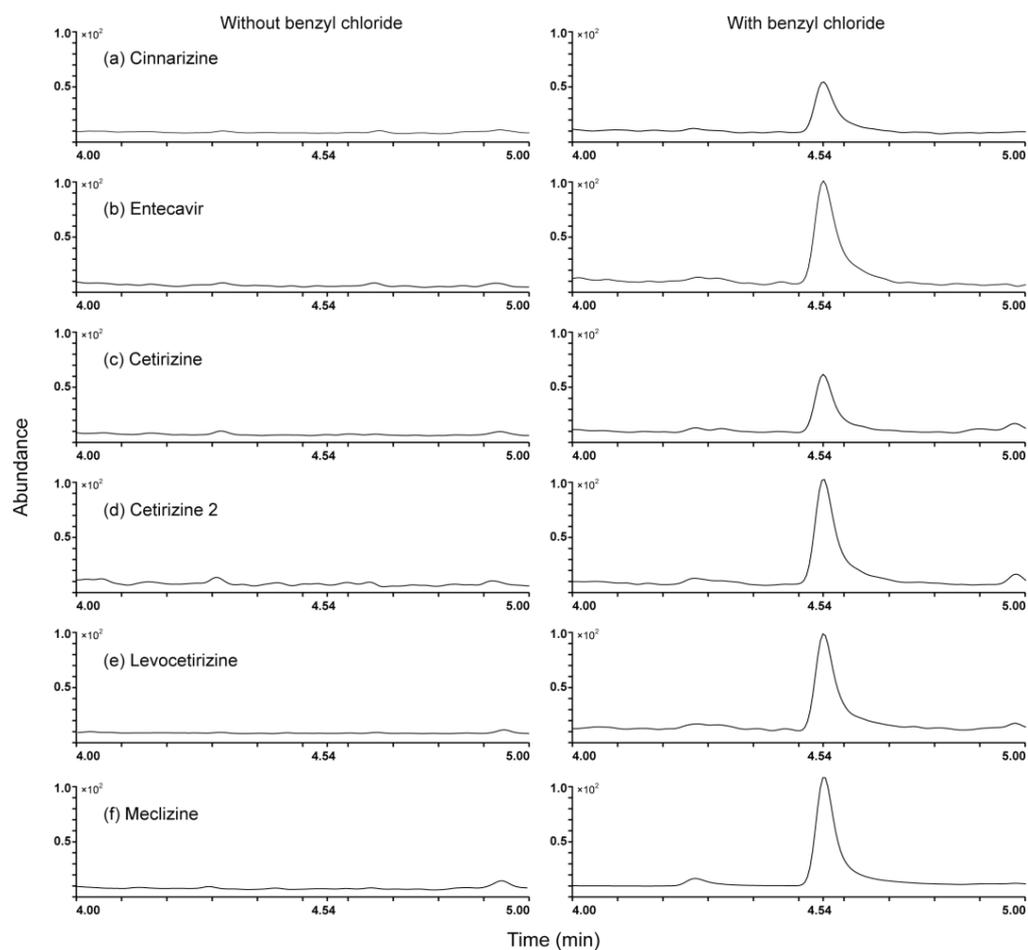


Figure 4. Ion chromatograms of six blank drug products and BC spiked drugs (0.1 $\mu\text{g}/\text{g}$) by solvent-free HS-GC/MS-SIM: (a) Cinnarizine, (b) Entecavir, (c) Cetirizine, (d) Centirizine 2, (e) Levocetirizine, and (f) Meclizine. The ion chromatograms in the left column are acquired without benzyl chloride, while those in the right column with benzyl chloride.

In contrast, the conventional GC-MS method exhibited lower sensitivity than the SF-HS-GC/MS method, when a known amount of benzyl chloride was added, as shown in Figure 2. Conversely, the solvent-free GC method demonstrated excellent sensitivity and reproducibility due to its lack of matrix interference and simple pre-treatment process without the use of organic solvents. These results validate the capability of the SF-HS-GC/MS method to effectively screen the presence of benzyl chloride in drug products.

3.5. Potential Limitations and Future Directions

In terms of potential limitations, we acknowledge a noteworthy aspect of the SF-HS-GC/MS method employed in this study, specifically regarding its vulnerability to temperature effects. It is essential to remain vigilant about the potential formation of unintended artifacts due to excessive temperature elevation during the analysis process. Despite these acknowledged limitations, it is important to emphasize that the overall analytical process benefits from streamlined sample preparation steps, which contribute to the method's convenience and expediency.

Expanding on the method's potential, we envision its applicability in the analysis of other volatile impurities within pharmaceuticals, particularly those impurities outlined in the ICH M7 guidelines. The ICH M7 (R1) guideline identifies a set of 14 impurities, including acrylonitrile, bis(chloromethyl) ether, 1-chloro-4-nitrobenzene, p-residine, dimethylcarbamoylchloride, ethyl chloride, and glycido, among others. We anticipate that the SF-HS-GC/MS method is well-suited for the analysis of most of these impurities, with the notable exceptions of bis(chloromethyl) ether, 1-chloro-4-nitrobenzene, and p-cresidine. These particular compounds possess the potential to generate hazardous vapors upon direct heating, thereby requiring careful consideration in the analytical process.

Furthermore, we emphasize the versatility of this method beyond its application to extended-release tablets. While our study primarily focused on this dosage form, we recognize that the SF-HS-GC/MS technique holds promise for adaptation to various pharmaceutical formats, including syrups and liquids. This adaptability underscores the method's broader relevance and potential impact on pharmaceutical quality control.

4. Conclusions

In this study, we successfully developed and validated a highly sensitive and efficient SF-HS-GC/MS method for the screening, and possibly the detection and quantitation, of benzyl chloride in APIs and drug products. The analytical method was rigorously validated following the Q2(R1) ICH guidelines, ensuring its reliability and reproducibility.

Our optimization efforts encompassed crucial parameters such as incubation temperature, incubation time, and sample amount, which yielded remarkable results using solvent-free split injection. The calibration curve of our method showcased outstanding linearity (>0.9998) across the concentration range of 0.05–5 $\mu\text{g/g}$, with the LOQ standing at a low 0.1 $\mu\text{g/g}$. This LOQ is significantly lower than the TTC level of 1.5 $\mu\text{g/day}$. Precision (%CV) consistently remained below 5%, and accuracy ranged between 95 and 105%.

The implementation of the solvent-free headspace technique offers several advantages over conventional HS-GC/MS and GC/MS methods. By eliminating the use of solvents, this method provides time-saving and convenience benefits. Furthermore, in pharmaceutical analysis where solvent interference effects can occur, it enables accurate and precise analysis without solvent impact.

Through careful optimization of key parameters, we successfully eliminated the possibility of artefactual formation of benzyl chloride during the screening process for benzyl chloride in cinnarizine. In conclusion, the findings of this study unequivocally demonstrate that the developed SF-HS-GC/MS method is highly effective in screening for the presence of benzyl chloride in various drug products without the need for solvents. This approach offers an accurate and reliable means of analysis compared with other commonly employed methods, while also saving time and minimizing solvent-based matrix effects. Thus, the SF-HS-GC/MS method presents a promising tool for the efficient screening of other volatile

hazardous impurities in drug products and APIs. Moreover, our developed method aligns with the current trajectory of pharmaceutical impurity management regulations, emphasizing the evaluation of potential impurity formation and implementation of strategic control measures.

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