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Trace Level Determination of 1-Chlorobutane in Candesartan Cilexetil Drug Substance

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Abstract
The 1-chlorobutanewas determined at the trace level by a capillary gas chromatographic method with the use of flame ionization detector in Candesartan Cilexetil (CAD). The column used for the development and validation was DB-624,60 m × 0.32 mm × 1.8 µm. The sample solution was prepared inN, N-Dimethylformamide (DMF); extracted with the help of headspace sampler and injected into chromatograph. The detection and quantitation limits obtained for the 1-chlorobutanewas1.9 ppm and 5.7 ppm, respectively. The method was validated according to the ICH (International Councilon Harmonization) guidelines. The linearity of the method was demonstrated, and the correlation coefficient was 0.99. The recoveries for the drug substance, candesartan cilexetilwas in the ranges of 97.2-102.1%. This is a robust method for the determination of 1-chlorobutane in active pharmaceutical ingredients (APIs).

Keywords
1-chlorobutane, gas chromatograph, flame ionization detector, method development, detection selectivity, headspace

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I. Introduction

Chloride of short-chain alkanes (n = 1–3) are potent carcinogens.Recently, increased attention has been paid to the health risksassociated with even trace amounts of chloromethane, chloroethane, 1-chloropropane, 2-chloropropane in drugs because of theirpotent mutagenic, carcinogenic, and teratogenic effects (1-4). These chlorides may be derived from excess starting material in pharmaceutical drugs or are formed as by-products in the reaction between hydrochloric acid (often used as a counter ion) and methanol, ethanol, 1-propanol, 2-propanol, (used as solvents in manufacturing process). The absorption of these compounds must be controlled to belowthe Threshold of toxicological concern (TTC) with respect to their maximum daily dosage(5-7). But our intention is to determine the content of 1-chlorobutane in CAD at trace level even toxicological study shows as negative results. Hence, it is imperative to develop analytical methods that are enough and meet all the regulatory requirements.

Since 1-chlorobutaneis a liquid at ambient temperature, with a boiling point around 77-78°C,they can be separated and quantifiedby gas chromatograph using theon-column injection technique. In this study, the 1-chlorobutane content was determined in CAD drug substance by means of headspace technique.

In this communication, we describe a simple and sensitive method for the determination of 1-chlorobutane inpharmaceuticalsusing capillary GC with Headspace sample andFlame Ionization Detector (FID) and no one has determined the 1-chlorobutane in Candesartan cilexetil drug substance. The method involves solid-liquid extractionusing headspace onto the columnassample introduction. Quantification was achieved using a single-point external standard calibration. The limits of detection and quantification (LOD, LOQ) of our method was1.9 ppm and 5.7 ppm with respect to 100 mg mL^{-1} of CAD.The study also includes method development and complete method validation.

II. Experimental

2.1. Chemicals

Standards for 1-chlorobutaneand N, N-Dimethylformamide were procured from Sigma Aldrich. Samples of CADwasprocured from Techno chemicals Ltd., India. The structures of 1-chlorobutane and CAD are shown in Fig.1.

2.2. Equipment

Method development and method validation were carried out using a GC 7890N, system equipped with headspace sampler G1888(Agilent Technologies, Singapore). The output signalswere monitored and processed using Empower software version 3, on anIntel i3 computer. The capillary GC columns used in this study were obtained from J&W Scientific (Santa Clara, CA, USA).

2.3. Preparation of solutions

Stock solutions of 1-chlorobutanewere prepared by transferring18mg of 1-chlorobutaneand diluting the solution to 100 mL in a volumetric flask with DMF. Standard solutions (18 μg mL^{-1}) wasprepared by further diluting1.0 mL of the stock solutions to 100 mLvolumetric flask with DMF.The CADsample solution was prepared by accurately weighing about 200mg of the sample into a 20 mL headspace vial and adding 2 mL of the DMF.

2.4. Chromatographic conditions

Amid polar of 6% cyanopropyl phenyl and 94% polydimethylsiloxane asstationary phase)-coated capillary column (0.32 mm x 60 m, 1.8 μm) was used for GC. Heliumgas used as carrier gas for GC at the flow rate of 3.0 mL min^{-1}. Hydrogen gas was used as Fuel at the flow rate of 30 mL min^{-1}, Zero air was used as oxidizer at the flow rate of450 mL $min^{-1}$and Helium was used as make up at the flow rate of 30 mL $min^{-1}$for FID.The GC oven temperature was firstmaintained at 45°C for 11min, raised to 175°C at the rate of 15 °C $min^{-1}$and held at this level for 2 min; further stepped up to 225°C at the 35°C min^{-1} and held at this level for 1 min and the total run-time of the method was 24.10 min. The headspace sampler conditions were the oven, loop, transfer-line temperatures 80°C, 175°C, 175°C respectively. The GC cycle time, vial equilibration time, vial pressure time, loop filling time, loop equilibration time, injection time were 30, 30, 0.3, 0.15, 0.05, 0.5 respectively. The vial pressure was 15 psi, the vial shaking mode was kept at high.The injector and detector (FID) were maintained at 200 °C and250 °C, respectively. Sample injection was carried out using an Agilent G1888 series headspace sampler.

III. Results and Discussion

3.1. Method development and optimization

The main challenge involved in the GC analysis of chloroalkaneson the stationary phase lies because of low boiling and shorter retaining times; so, achieving the desired detection and quantification limits using 60 m column length, the most commonly available instrument, i.e., a gas chromatograph coupled headspace configured with an FID.Thus, amid-bore capillary column (0.32 mm I.D.) with a high-capacity bonded stationary phase would be the obvious choice. An appropriate initial column temperature in combination with a moderate inlet temperature (200°C) may allow for a relatively large injection volume without significant deterioration in column efficiency.

The effects of concentration on the separation and quantification of 1-chlorobutanewas investigated by injecting 4 μL of the standard solution and sample solution leads to the blunt peak shape and results in the failure of precision were might due to the creation the air bubbles or gaps during the on-column injection. Further studies were not performed to determine the maximum injection volume because the satisfactory peak area with the precise and accurate values were obtained; moreover, higher injection volume may affect the peak shapes.A headspace sampler technique has been used in this study to avoid the overload of the sample onto the column and for getting the precision acceptance criteria (%RSD should be not more than 15.0%).The temperature allowed baseline separation of the 1-chlorobutane, and other residual solvents used in the synthetic process of CAD from interfering peaks.

Ourmethod utilizes an extraction and injection approach for the solid-liquid extraction analysis. Several factors were considered for the selection of thesample solvent, including purity, extraction ability, and chemical compatibility with the compounds of interest. Purity of the sample solvent plays a critical role in the detection oflow concentrations (ppm) of chloroalkanes. We found that HPLC-grade solvents generally allow for interference-free analysis. 1,3-Dimethyl imidazolidinone (DMI), dimethyl sulfoxide (DMSO), and benzyl alcohol, which are used in residual solvent analysis, are incompatible for use with the extraction. Hence, it is preferable touse apolar solvent such as N, N-Dimethylformamide. The data revealthat the present method isvery highly sensitive for the determination of 1-chlorobutane in CAD APIs. The limitation of the method isthat only a 60 m column length,and not a 30 m column length, would be suitable for shorter run-time.

3.2. Method validation

Validation of our method was conducted according to the ICHguidelines(8). The validation parameters were specificity, accuracy, precision, sensitivity, linearity, robustness, ruggedness, and solution stability. In the pharmaceutical industry, the LOQ is defined as the lowest amount of analyte in a sample that can be

quantitatively determined with suitable precision and accuracy. Based on the precision and accuracy data, theLOQ was determined to be less than or equal to 5.7 ppm for 1-chlorobutane. The results for system suitability and precision are given in TableI.

The linearity of the method was determined by preparing and analyzing a series of 7 standard solutions to cover the concentration range of the 5.7 to 38 ppm for 1-chlorobutane.The linearity graphs for1-chlorobutaneare shown in Figs. 2. The precision resultsfor the LOQ are given in Table II. Regression analysis of the peak area versus concentration data yields an $r^2 > 0.99$ for each of the three calibration curves, as shown in Table II.

The experimental resultsalso indicate the excellent precision of this method, even without the use of an internal standard. Multiple injections were performed for the 1-chlorobutane standard solutions. For six injections of the standard solution, the RSD was less than1%.An Accuracy of the method was determined by analyzing drug samples spiked with known concentrations of 1-chlorobutane. The spiked levels were at 5.7, 18, and 38 μgmL^{-1}.The recovery was in the range 97.2-102.1%. The recovery results for CADare given in TablesIII. Because this method is based on the solid-liquid extraction and injection approach, accumulation of the drug in the injection liner, which negatively impact recovery, is avoided. Therefore,it is not necessary to replace the injection liner after every sequence of injections. The method was also validated in terms of solution stability at room temperature. A standard solution of 1-chlorobutane at a concentration of $18\mu gmL^{-1}$was injected at regular intervals upto 7 days at room temperature. The recovery was in the range of 97-102%, confirming solution stability. The GC chromatograms for blank, standard, sample, and spiked sample at LOQ, and 100%level solutions are shown in Fig. 4-7.

IV. Conclusion

A simple and sensitive method based on GC has been developed and validated (according to the ICH guidelines) for the trace analysis of 1-chlorobutanein pharmaceutical drugs. The FID used in this method is readily available in most of the testing laboratories in the pharmaceutical industry and is relatively simple to use. The LOD and LOQ of our method are 1.9 ppm, and 5.7ppm, respectively, for 1-chlorobutanein the drugs CAD.Based on the results of the study, we expect this method to be useful for the routine analysis of the aforesaid impurities in APIs.

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None
Conflicts of interest:
None.

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TableI. System suitability and precision

% RSD of area response for 100% standard	
Injection	1-Chlorobutane
%RSD	0.93
Criteria	Not more than 15%
% RSD of retention time	
Injection	1-Chlorobutane
% RSD	0.0
Criteria	Not more than 0.5%
Tailing factor	
Injection	1-Chlorobutane
1	1.03
Criteria	NMT 2.0
USP Plate counts	
Injection	1-Chlorobutane
1	280394
Criteria	NMT 2.0

Table II. Summary of linearity results

Level (% w.r.t target level concentration)	Standard Concentration (µg/mL)	Peak area
30	5.6934	2.4
40	7.5913	3.3
80	15.1825	6.7
100	18.9782	8.5
120	22.7738	10.2
160	30.3650	13.2
200	37.9563	16.9
Slope		0.4451
Intercept		-0.0671
R^2		0.9994
Observation		7

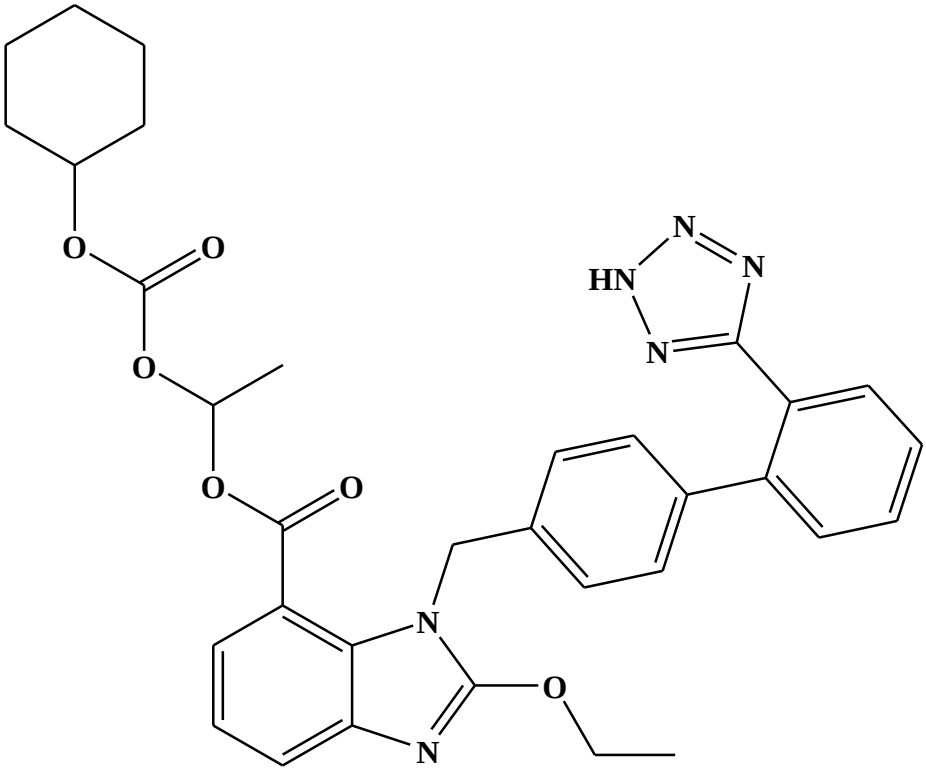
Table III. Accuracy results for 1-Chlorobutanedetection in Candesartan cilexetil

%level	**% Recovery**	
	Sample #	1-Chlorobutane
QL	1	97.2
	2	97.2
	3	97.2
	Mean	97.2
100	1	99.6
	2	102.1
	3	99.6
	Mean	100.4
150	1	100.9

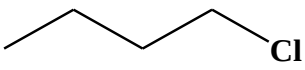
2	100.9
3	100.2
Mean	100.6

Figure Legends

Fig. 1. Structures of Candesartan cilexetil and 1-Chlorobutane



Candesartan cilexetil



1-Chlorobutane

Fig. 2. Linearity graph for 1-Chlorobutane

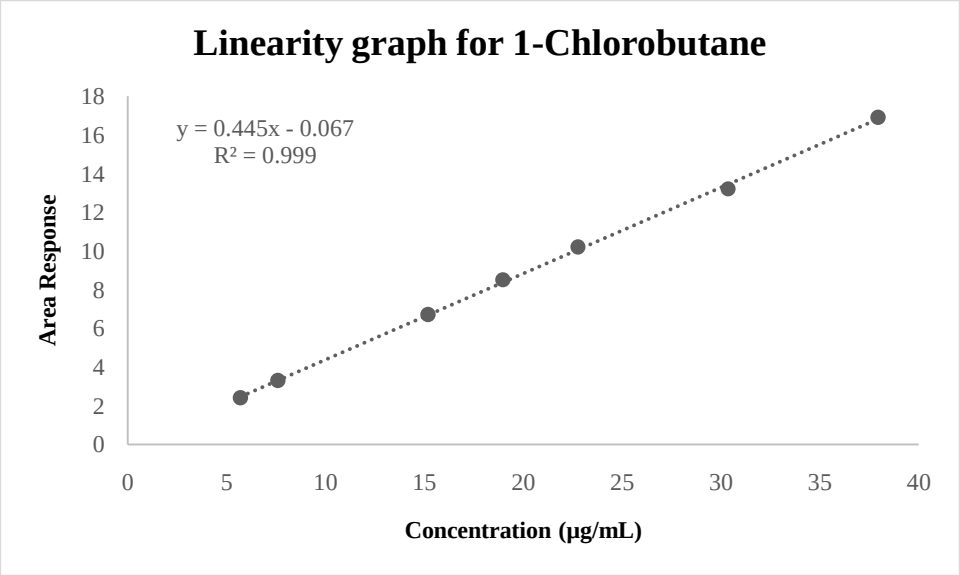
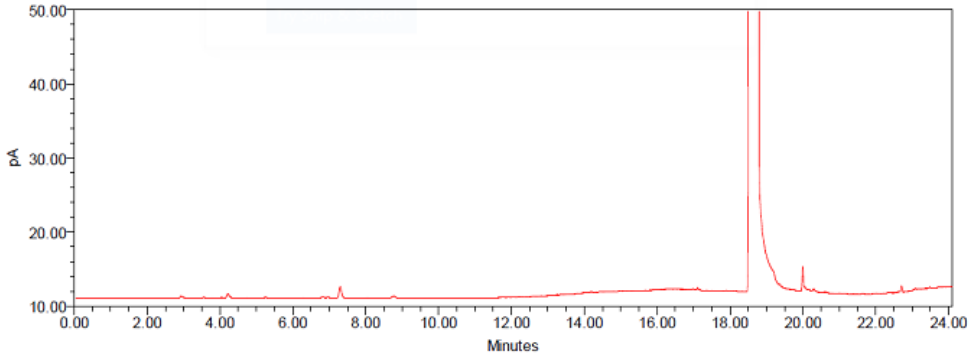


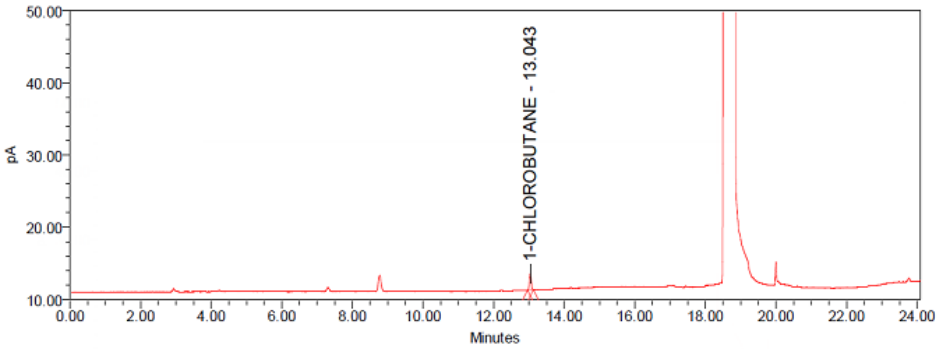
Fig. 3. Gas chromatogram of blank solution



Peak Results

	Name	RT	Area	% Area
1	1-CHLOROBUTANE	13.043		

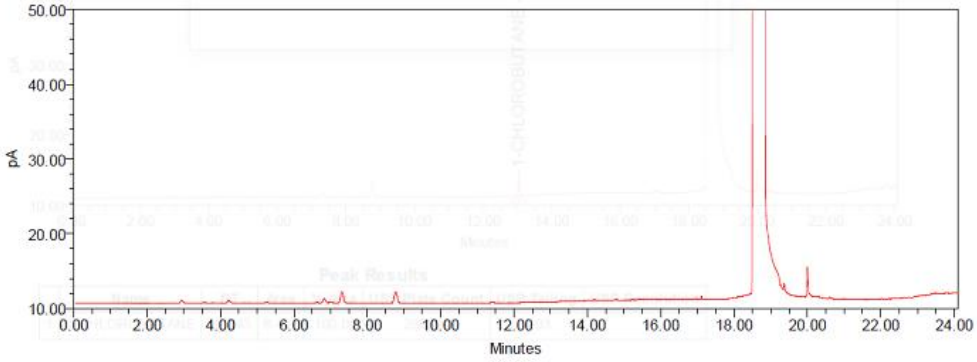
Fig. 4. Gas chromatogram of 1-Chlorobutane at 100% standard solution



Peak Results

	Name	RT	Area	% Area	USP Plate Count	USP Tailing	USP Resolution
1	1-CHLOROBUTANE	13.043	8.401	100.00	280394	1.03	

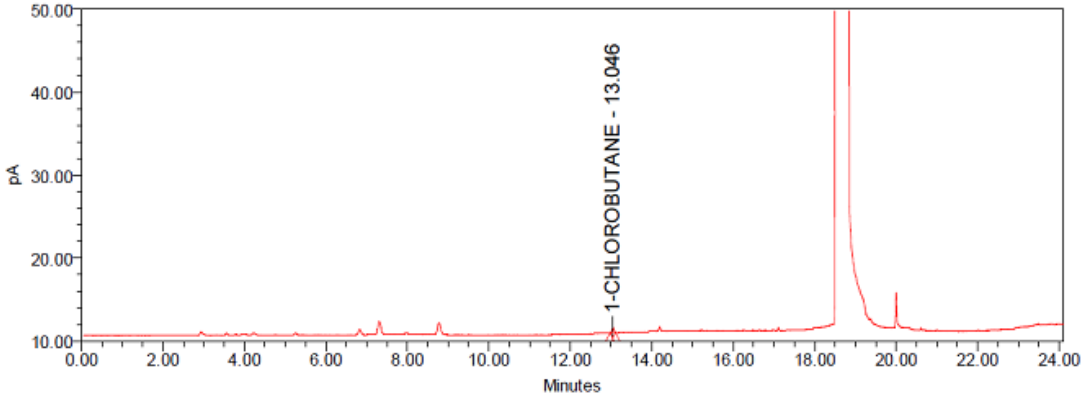
Fig. 5. Gas chromatogram of CAD sample solution



Peak Results

	Name	RT	Area	% Area
1	1-CHLOROBUTANE	13.043		

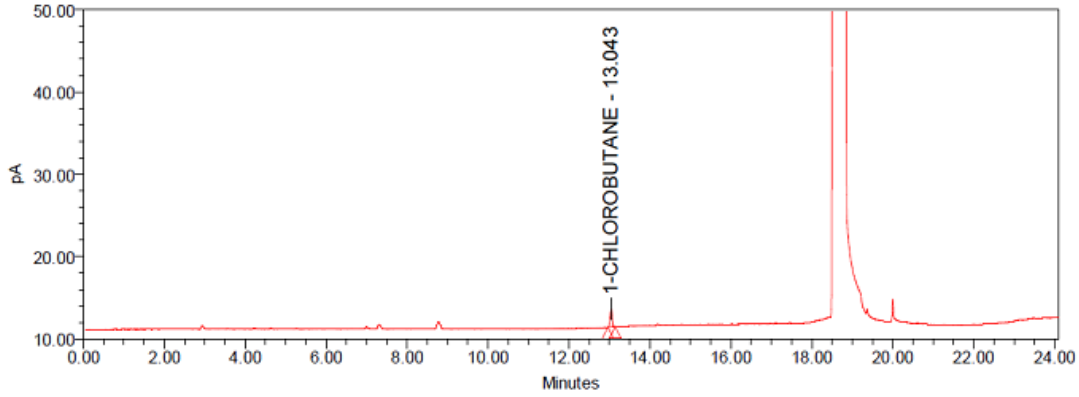
Fig. 6. Gas chromatogram of Spiked samplesolution at LOQ level of 1-chlorobutane



Peak Results

	Name	RT	Area	% Area
1	1-CHLOROBUTANE	13.046	2.3	100.000

Fig. 7. Gas chromatogram of Spiked samplesolution at 100%level of 1-chlorobutane



Peak Results

	Name	RT	Area	% Area
1	1-CHLOROBUTANE	13.043	8.2	100.000