TRACE QUANTIFICATION OF EPICHLOROHYDRIN AS A POTENTIAL GENOTOXIC IMPURITY IN ACEBUTOLOL HYDROCHLORIDE DRUG SUBSTANCE BY ADVANCED GAS CHROMATOGRAPHY TECHNIQUE.

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Abstract

Epichlorohydrin is a potentially genotoxic impurity widely used as an intermediate in the synthesis of several pharmaceutical active ingredients. Due to its mutagenic and carcinogenic potential, its presence in drug substances must be controlled at trace levels in accordance with regulatory requirements. The present study describes the development and validation of a sensitive, accurate, and reliable headspace gas chromatographic method coupled with flame ionization detection (HS-GC-FID) for the determination of epichlorohydrin in Acebutolol Hydrochloride API. Chromatographic separation was achieved using an Rtx-1301 capillary column under optimized headspace and gas chromatographic conditions. The developed method was validated according to ICH guidelines for specificity, system precision, method precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), and intermediate precision. The method exhibited excellent specificity with no interference at the retention time of epichlorohydrin. Precision studies demonstrated satisfactory reproducibility with %RSD values well within acceptance limits. Accuracy studies showed recoveries ranging from 93% to 112%, confirming the reliability of the method. The method was linear over the studied concentration range with a correlation coefficient of 0.981. The LOD and LOQ values were found to be 0.7 ppm and 1.5 ppm, respectively, indicating adequate sensitivity for trace-level determination. The validated HS-GC-FID method is simple, precise, accurate, and suitable for routine quality control monitoring of epichlorohydrin in Acebutolol Hydrochloride API.

1.INTRODUCTION

Genotoxic impurities are chemical substances that can interact with genetic material and induce mutations, chromosomal damage, or carcinogenic

effects even at very low concentrations. Their presence in pharmaceutical products poses a significant risk to patient safety, making their identification, control, and quantification an important aspect of pharmaceutical quality assurance[1]. Regulatory agencies such as the International Council for Harmonisation (ICH) have established stringent guidelines for the assessment and control of genotoxic impurities in drug substances and drug products. According to ICH M7 guidelines, potentially mutagenic impurities must be controlled at trace levels based on acceptable daily exposure limits to minimize the risk of cancer in patients[2]. Acebutolol Hydrochloride is a cardio selective β_1-adrenergic receptor blocking agent widely used in the management of hypertension, angina pectoris, and cardiac arrhythmias[3]. The synthesis of Acebutolol Hydrochloride involves several chemical intermediates and reagents, among which epichlorohydrin plays a critical role as a starting material or synthetic intermediate[4]. Epichlorohydrin is a reactive chlorinated epoxide compound that has been classified as a probable human carcinogen due to its mutagenic and genotoxic potential. Consequently, residual traces of epichlorohydrin remaining in the active pharmaceutical ingredient (API) must be carefully monitored and controlled to ensure compliance with regulatory requirements and patient safety standards[5]. Due to its volatile nature and the requirement for determination at trace levels, highly sensitive analytical techniques are necessary for the quantification of epichlorohydrin in pharmaceutical substances. Headspace gas chromatography coupled with flame ionization detection (HS-GC-FID) is widely recognized as an effective analytical technique for the determination of volatile organic impurities because of its excellent sensitivity, selectivity, and reproducibility[6]. The headspace sampling approach minimizes matrix interference and enhances the detection of volatile compounds present at low concentrations[7]. The present study focuses on the development and validation of a simple, sensitive, and reliable headspace gas chromatographic method for the determination of epichlorohydrin as a genotoxic impurity in Acebutolol Hydrochloride API[8-10]. The method was validated according to ICH guidelines with respect to specificity, precision, accuracy, linearity, limit of detection, limit of quantification, ruggedness, and robustness to demonstrate its suitability for routine quality control analysis.

2.EXPERIMENTAL

2.1 Materials and Reagents

Acebutolol hydrochlorideAPI, Epichlorohydrin and 1,4 – Dioxane standards were obtained from Sigma Aldrich. Dimethyl acetamide (DMA)was used for preparation of diluent and sample solutions.

2.2 Instrumentation

Chromatographic analysis was performed using AgilentGC 7890 A system equipped with a FID detector. Data acquisition and processing were carried out using Empower software.

2.3 Chromatographic Conditions

The chromatographic analysis was performed using an Agilent Gas Chromatograph 6890N

Column – Rtx-1301,30m X 0.53 mm,3.0µm

equipped with:

Oven

Initial temperature-80ºC, Initial time-7.0 min, Ramp-10ºC/min, Final temperature-150ºC

Hold time-10 min, Runtime-24 min, Carrier gas: Helium

Injector/Inlet

Injector temperature-150°C, Split ratio-1:5, Carrier gas-Helium, Carrier gas flow-2.7 ml/min

Detector

Detector-FID, Detector Temperature-240ºC, Hydrogen flow-40 ml/min, Airflow-400ml/min

Headspace Conditions

Vial oven temperature: 60°C, Loop temperature: 60°C, Transfer line temperature: 100°C

Vial equilibration time: 15 min, Injection time: 1.0 min

2.4 Blank Preparation

Dimethyl acetamide.

2.5 Standard Preparation

Preparation of Internal Standard Solution: Transferred 100 µL of 1,4-Dioxane into a 100 mL volumetric flask containing about 20 mL of diluent. The solution was diluted to volume with diluent and mixed well.

Preparation of Epichlorohydrin Stock Standard Solution: Transferred 250 µL of Epichlorohydrin (density of 1.183 g/mL, about 296 mg of Epichlorohydrin) into a 100 mL volumetric flask. The solution was diluted to volume with diluent and mixed well.

Preparation of Epichlorohydrin Stock Standard Solution-1: Pipetted 10.0 mL of Epichlorohydrin Stock Standard Solution into a 50 mL volumetric flask. The solution was diluted to volume with diluent and mixed well.

Preparation of Epichlorohydrin Pre-Mix Standard Solution: Pipetted 10.0 mL of Epichlorohydrin Stock Standard Solution into a 50 mL volumetric flask. The solution was diluted to volume with diluent and mixed well.

Standard Preparation in Headspace Vial: Approximately 2000 mg of sample was accurately weighed into a headspace vial. Subsequently, 50 µL of Epichlorohydrin Pre-Mix Standard Solution and 3.0 mL of Milli-Q water were added to the same headspace vial, and the vial was crimped. The resulting concentration of Epichlorohydrin was about 7 ppm with respect to the sample concentration.

2.6 LOD Solution: Pipetted 0.75 mL of Epichlorohydrin Stock Standard Solution into a 50 mL volumetric flask. The solution was diluted to volume with diluent and mixed well. Then, 5.0 mL of the above solution was transferred into a 10 mL volumetric flask and diluted to volume with diluent and mixed well. Approximately 2000 mg of sample was accurately weighed into a headspace vial. Subsequently, 50 µL of the prepared LOD solution and 3.0 mL of Milli-Q water were added to the same headspace vial, and the vial was crimped. The resulting concentration of Epichlorohydrin was about 0.5 ppm with respect to the sample concentration.

2.7 Blank Preparation in Headspace Vial: Transferred 25 µL of internal standard solution into a headspace vial. Subsequently, 3.0 mL of Milli-Q water was added, and the vial was crimped.

2.8 Sample Preparation

Approximately 2000 mg of sample and add 25µL of internal standard solution and pipetted out 3.0 ml of Milli Q water into a headspace vial and crimped it.

3. Method Validation

3.1 System Precision

A standard solution was prepared as per the method and injected. The %RSD for peak area response from three (3)-replicate injections of the standard solution were calculated resolution between Epichlorohydrin and 1,4 – Dioxane were calculated. The % RSD for peak areas should be NMT 10% and USP resolution between Epichlorohydrin and 1,4 – Dioxane should be NLT 2.

3.2 Method Precision

Precision of the method was estimated by performing repeatability. Method precision was determined by injecting six (6) individual samples of Acebutolol Hydrochloride spiked with Epichlorohydrin at the specification level,

prepared as per the method.The %RSD of Epichlorohydrin content obtained from six (6) sample preparations should be not more than (NMT) 25.0%.

3.3 Accuracy

Accuracy studies were performed by known amount of Epichlorohydrinat the levels of LOQ,100% and 130% of the standard theoretical concentration. The % Recovery should be between 70%-130%. The over all % RSD should be NMT 15.0%.

3.4 Linearity

Linearity of the method was established by injecting the standard at theoretical concentration in different level from LOQ% to 160%. The correlation coefficient square should be NLT 0.97.

3.5 Specificity

The Chromatograms of standard, sample and diluent of were compared. No Interference should be observed at the retention time of Epichlorohydrin.

3.6 Precision at LOQ Level

Three (3) replicates of LOQ solution preparation were injected into GC system. The %RSD for peak responses from three (3)-replicate injections of the LOQ solution were calculated. The % RSD for peak areas NMT 25%.

3.7 Limit of Quantification (LOQ) and Precision at LOQ

Serially diluted Epichlorohydrin standard solution to lower levels and determined the LOD & LOQ values by signal to noise ratio method. The Signal to noise ratio for LOD should be NLT 2 and for LOQ NLT 5.

3.8 Intermediate Precision

The method precision ruggedness (reproducibility) of content of theEpichlorohydrin was determined by preparing standard and six (6)-individual samples spiked with Epichlorohydrinat the specification level by a second analyst on a different day.Samples were prepared as per the method. The %RSD for the content of Epichlorohydrinfrom six (6) sample preparations should be NMT 25.0%. The % difference in content of Epichlorohydrinbetween Method precision and Intermediate precision results should be NMT 25.0%.

4. RESULTS AND DISCUSSION

4.1 System Precision

System precision evaluates the repeatability and performance of the chromatographic system by multiple injections of the standard solution under identical operating conditions. It is assessed by calculating the percentage relative standard deviation (%RSD) of peak responses and verifying chromatographic parameters such as resolution. A low %RSD indicates consistent detector response and stable instrument performance. As shown in Table 1, the %RSD obtained from three replicate

injections was 6.61%, which complies with the acceptance criterion of NMT 10.0%. The USP resolution between Epichlorohydrin and 1,4-Dioxane was found to be 6.6, which is well above the required limit of NLT 2.0. These results demonstrate that the chromatographic system is precise and capable of producing reproducible responses.

Table1: System Precision Results

S.No	Sample Name	Response	USP Resolution
01	Standard -1	1.245455	6.6
02	Standard -2	1.254898	
03	Standard -3	1.112565	
Average		1.204306	
% RSD		6.61	

4.2 Method Precision

Method precision evaluates the repeatability of the analytical method by analysing multiple sample preparations under identical conditions. As shown in Table 2, the %RSD of Epichlorohydrin content from six sample preparations was 10.89%, which met the acceptance criterion of NMT 25.0%. Therefore, the method was found to be precise.

Table2: Method Precision Results

Sample Name	Epichlorohydrin (ppm)
Method Precision -01	2.4
Method Precision -02	2.1
Method Precision -03	2.1
Method Precision -04	2.1
Method Precision -05	2.0
Method Precision -06	1.7
Average	2.1
% RSD	10.89

4.3 Recovery study

Accuracy assesses the closeness of the measured value to the true value and is determined through recovery studies. The recovery results presented in Table 3 were within the acceptance range of 70–130%, with an overall recovery of 98% and %RSD of 12.8%. Hence, the method was considered accurate.

Table3: Method Accuracy Results

% Level	% Recovery* Average	%RSD	Sample Name
Accuracy - LOQ	112	4.3	Epichlorohydrin
Accuracy-100%	93	15.0	
Accuracy-130%	95	3.5	
Overall % Recovery	98	12.8	

*** Average of triplicate preparations**

*** Accuracy 100% - Average of six preparations**

4.4 Linearity

Linearity determines the proportional relationship between analyte concentration and detector response. As shown in Table 4, the correlation coefficient (r^2) was 0.981, meeting the acceptance criterion of NLT 0.97. Therefore, the method exhibited good linearity over the studied concentration range.

Table4: Linearity Results

Name	Epichlorohydrin	
	Concentration (ppm)	Peak area Response
LOQ	1.4588	0.302154
Linearity-83%	1.8489	0.328587
Linearity-100%	2.2125	0.378874
Linearity-130%	2.9256	0.520589
Linearity-160%	3.6687	0.598987
Correlation-Co efficient	0.981	

4.5 Specificity

Specificity evaluates the ability of the method to measure the analyte without interference from other components. The results in Table 5 showed no interference at the retention time of Epichlorohydrin. Hence, the method was found to be specific.

Table5: Specificity Results

Solvent Name	Blank	RT of Individuals
Epichlorohydrin	**ND**	**9.268**
1,4 -Dioxane	**ND**	**8.273**
Acetone	ND	3.819

4.6 Precision at LOQ

Precision at LOQ verifies the reproducibility of the method at the lowest quantifiable concentration. As shown in Table 6, the %RSD was 4.81%, which is within the acceptance criterion of NMT 25.0%. Therefore, the method was precise at the LOQ level.

Table6: Precision at LOQ Results

Sample Name	Epichlorohydrin Peak area response
LOQ Precision -01	0.287856
LOQ Precision -02	0.265487
LOQ Precision -03	0.289782
Mean	0.281042
%RSD	4.81

4.7 LOD and LOQ

LOD and LOQ indicate the sensitivity of the analytical method. The LOD and LOQ values shown in Table 7 were 0.7 ppm and 1.5 ppm, respectively, demonstrating adequate sensitivity for trace-level determination of Epichlorohydrin.

Table 7: LOD & LOQ Results

Sample Name	LOD (S/N)	LOQ (S/N)
Epichlorohydrin	5	8
	Concentration	**Concentration**
	0.7 ppm	1.5

4.8 Intermediate precision

Intermediate precision evaluates the reproducibility of the method under normal laboratory variations. The %RSD obtained in Table 8 was 4.98%, which met the acceptance criterion of NMT 25.0%. Therefore, the method was found to be rugged and reproducible. Analyst difference assesses consistency between results generated by different analysts. As shown in Table 9, the percentage difference between Analyst-1 and Analyst-2 was 5.0%, which is within the acceptance criterion. Hence, the method demonstrated good analyst-to-analyst reproducibility.

Table8: Intermediate Precision Results

Sample Name	Epichlorohydrin (ppm)
Intermediate Precision -01	2.3
Intermediate Precision -02	2.2
Intermediate Precision -03	2.2
Intermediate Precision -04	2.2
Intermediate Precision -05	2.3
Intermediate Precision -06	2.0
Average	2.0
% RSD	4.98

Table 9: Analyst Difference

Name	Analyst-1	Analyst-2	% Difference
Epichlorohydrin	2.1	2.0	5.0

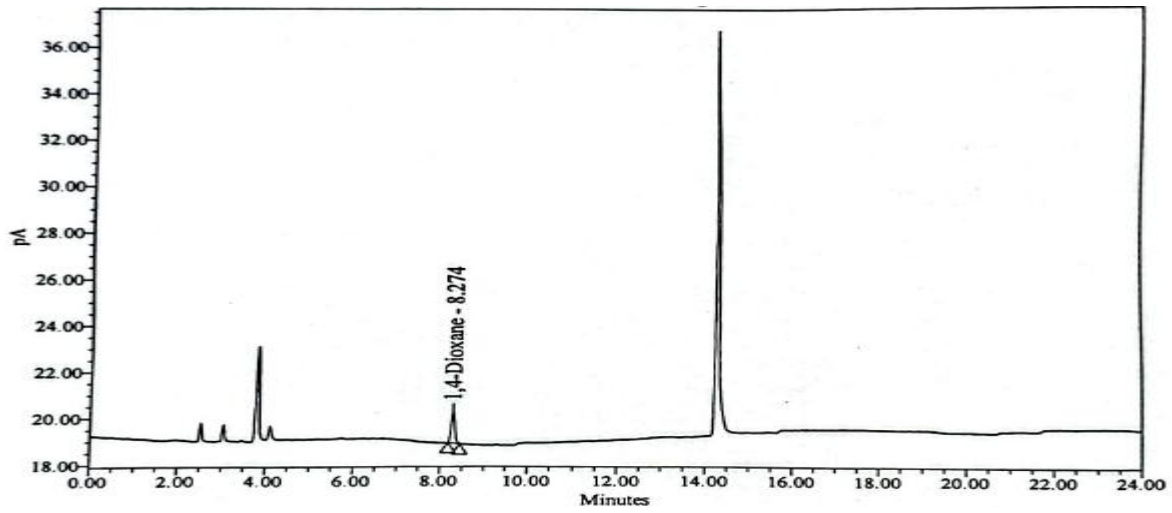

Figure 1. Chromatogram for Blank

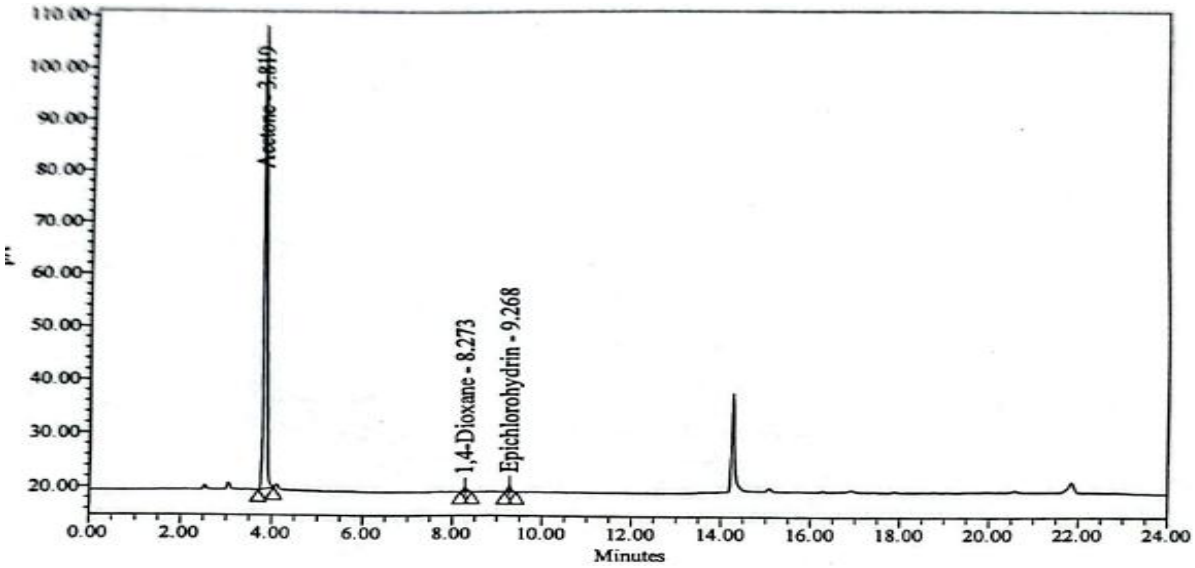

Figure 2. Chromatogram for Standard

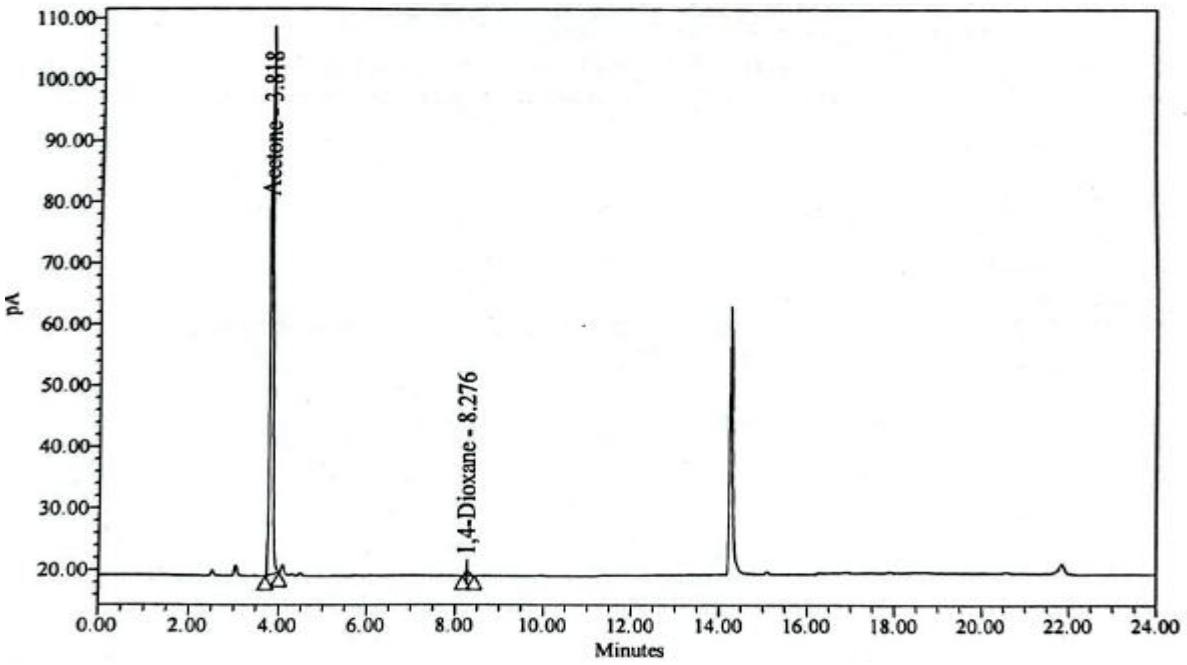

Figure 3. Chromatogram for control sample

5.CONCLUSION

A simple, sensitive, and reliable headspace gas chromatographic method coupled with flame ionization detection (HS-GC-FID) was successfully developed and validated for the determination of epichlorohydrin in Acebutolol Hydrochloride API. The method demonstrated excellent specificity with no interference observed at the retention time of epichlorohydrin. Validation studies confirmed satisfactory system precision, method precision, accuracy, linearity, sensitivity, and intermediate precision in accordance with ICH guidelines. The %RSD values obtained for precision studies were within the established acceptance criteria, indicating good reproducibility of the method. Recovery studies demonstrated acceptable accuracy across the evaluated concentration levels, while the correlation coefficient of 0.981 confirmed the linearity of the analytical procedure. The low LOD and LOQ values further established the capability of the method to detect and quantify trace levels of epichlorohydrin. In addition, the method showed good ruggedness and reliability under normal laboratory conditions. Therefore, the developed HS-GC-FID method is suitable for routine quality control analysis and regulatory compliance testing of epichlorohydrin as a genotoxic impurity in

Acebutolol Hydrochloride API, thereby ensuring product quality and patient safety.

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