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Enantiomeric Separation of S-Epichlorohydrin and R-Epichlorohydrin by Capillary Gas Chromatography with FID Detector

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Abstract

The aim of this study was to develop a simple and derivatization free method for the Quantification of S-Epichlorohydrin in R-Epichlorohydrin by using a gas chromatography coupled with flame ionization detector (FID). Enantiopure epichlorohydrin was a valuable epoxide key starting material for preparing optically active Rivaroxaban. The enantiomeric separations of S-Epichlorohydrin and R-Epichlorohydrin were achieved on Gamaa-Dex-225 (30 meters × 0.25 mm I.D, 0.25 μm) column with a total run time of 30 min. Nitrogen was used as a carrier gas with constant pressure 25.0 psi. The critical experimental parameters such as, column selection, flow rate, injection volume and diluent were studied and optimized. Excellent correlation coeffient between peak responses and concentrations was >0.9998. The recoveries of S-Epichlorohydrin spiked in R-Epichlorohydrin were in the range from 98.2% to 102.8%. Limit of quantitation for S-Epichlorohydrin was sufficiently lower than limits specified by ICH. The method has validated as per International Conference on Harmonization (ICH) guidelines. A precise, accurate, linear and robust Gas Chromatography method was developed for the quantification of S-Epichlorohydrin in R-Epichlorohydrin for Rivaroxaban.

Keywords

S-Epichlorohydrin, R-Epichlorohydrin, Method Development, ICH Guidelines, Method Validation, Gas Chromatography

1. Introduction

Separation of the enantiomers of chiral drugs has become an important issue in analyt-

ical chemistry in recent years, because of differences in the biological activity and pharmacokinetic properties of drug enantiomers [1] [2]. Epichlorohydrin is an organochlorine compound and it is a chiral molecule and exists as R-Epichlorohydrin and S-Epichlorohydrin mentioned in **Figure 1(a)** and **Figure 1(b)**. Epichlorohydrin is a versatile precursor in the synthesis of many organic compounds. Epichlorohydrin is a colorless, volatile and highly reactive liquid. It is soluble in most organic solvents and slightly soluble in petroleum hydrocarbons and in water [3]. Epichlorohydrin (1-chloro-2,3-epoxypropane) was used mainly for the manufacture of pharmaceutical products, glycerol, unmodified epoxy resins and to a lesser extent elastomers, water-treatment resins, surfactants, ion exchange resins, plasticizers, dyestuffs, oil emulsifiers, lubricants, and adhesives [4]. Epichlorohydrin has been classified as a probable carcinogen (group A) to human by the International Agency for Research on Cancer (IARC) [5].

Rivaroxaban is chemically 5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-orpholinyl) phenyl]-1,3-oxzolidin-5-yl}methyl)-2-thiophene-carboxamide with molecular formula $C_{19}H_{18}ClN_3O_5S$. Rivaroxaban was used for potent anticoagulant and antithrombotic effects [6] [7]. Rivaroxaban was approved by US Food and Drug Administration (FDA). Gas chromatography is the most commonly used technology for the analysis of Epichlorohydrin. From the literature review there were few analytical methods which have been reported for Epichlorohydrin such as GC methods [8] [9] [10] [11] [12] and GC-MS methods [13] [14]. There was no reported method for the determination of S-Epichlorohydrin in R-Epichlorohydrin by gas chromatographic method for Rivaroxaban. The major objective of the present work is to develop a simple and robust GC method for determination of S-Epichlorohydrin in R-Epichlorohydrin for Rivaroxaban. Hence, a reproducible gas chromatography with FID detector method was developed for the quantitative determination of S-Epichlorohydrin in R-Epichlorohydrin. This method was successfully validated according to the International Conference Harmonization (ICH) guidelines (Validation of Analytical Procedures: Test and Methodology Q2).

2. Experimental

2.1. Materials and Reagents

R-Epichlorohydrin and S-Epichlorohydrin were purchased from Sigma-Aldrich Dich

(a)   (b)
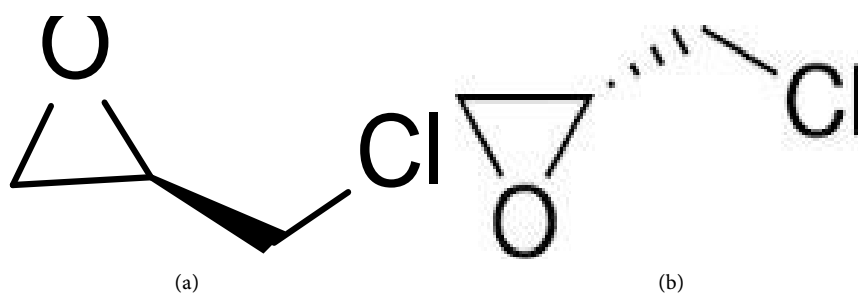
Figure 1. (a) Structures of R-Epichlorohydrin; (b) Structures of S-Epichlorohydrin.

loromethane was purchased from Merck, Germany.

2.2. Instruments and Software

A calibrated electronic single pan balance Mettler Toledo. All analysis performed on Agilent 6890 and 7890 modules equipped with FID detectors. Empower-3 software was used for signal monitoring and data processing. Microsoft Excel 2007 was used for analysis of validation results.

2.3. Chromatographic Conditions

The method was developed by using Gamaa-Dex-225 (30 meters × 0.25 mm I.D, 0.25 μm) column. The separation was achieved using an isothermal oven program 50˚C for 30 min. The injector temperature was maintained at 250˚C. Nitrogen was used as a carrier gas with constant pressure 25.0 psi. The detector temperature was maintained at 250˚C. The injection volume was 1.0 μL. Split ratio 1:50 and Runtime was 30.0 min.

Dichloromethane was used as diluent during the standard and test samples preparation. Chromatograms were summarized in Figures 2(a)-(d).

2.4. Preparation of Solutions

2.4.1. Preparation of S-Epichlorohydrin Stock Solution
Transfer accurately 0.1 mL of S-Epichlohydrin standard in 50 mL volumetric flask, containing 10.0 mL diluent and made up to volume with diluent.

2.4.2. Preparation of System Suitability Solution
Transfer accurately 1.0 mL of standard in 50 mL volumetric flask, containing 10.0 mL diluent and to this add accurately 0.5 mL of above S-Epichlorohydrin stock solution and made up to volume with diluent.

2.4.3. Preparation of Standard Solution
Transfer accurately 1.0 mL of standard in 50 mL volumetric flask, containing 10.0 mL diluent and made up to volume with diluent.

2.4.4. Sample Solution Preparation
Transfer accurately 1.0 mL of test sample in 50 mL volumetric flask, containing 10.0 mL diluent and made up to volume with diluent.

3. Method Validation

The method has been validated by GC as per ICH guidelines [15]. The method was validated for the following parameters: Precision, Linearity, Accuracy, Robustness, Solution stability, Limit of Detection, Limit of Quantification and Ruggedness.
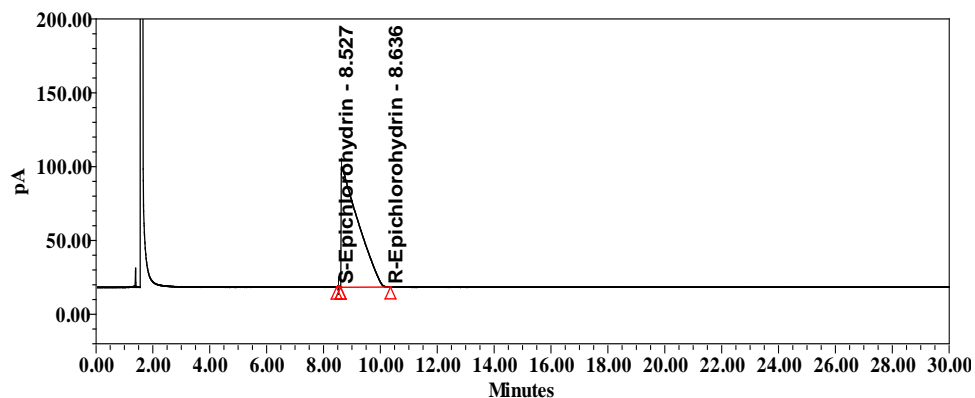
3.1. Precision

The precision of the method was verified by repeatability and by intermediate precision. Repeatability of the method was checked by (Agilent 7890 module equipped with

(a)

(b)

(c)

(d)
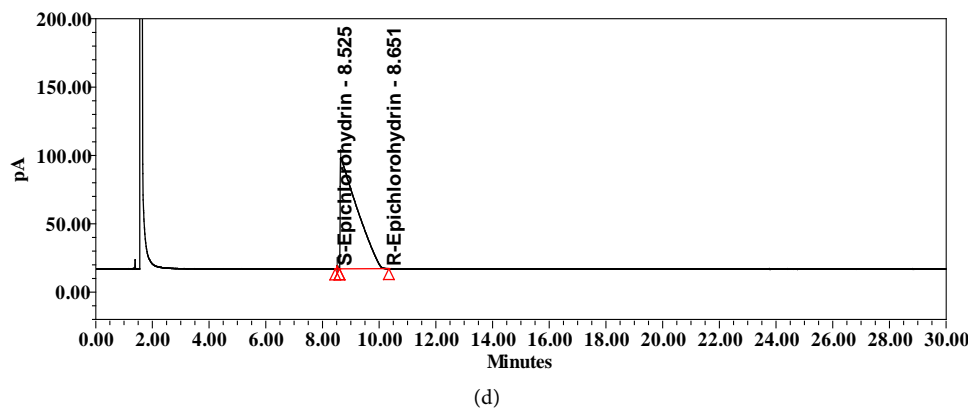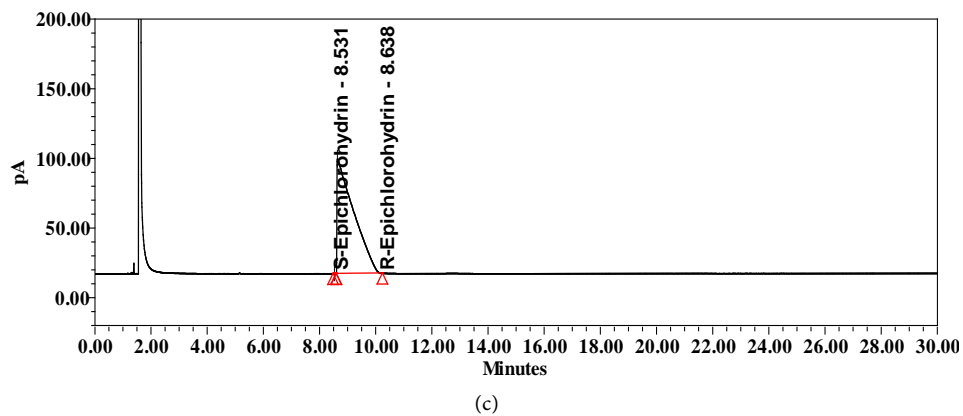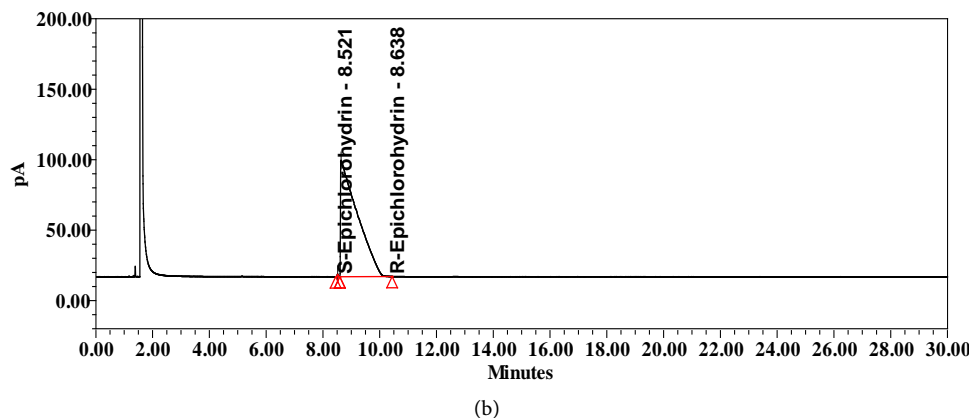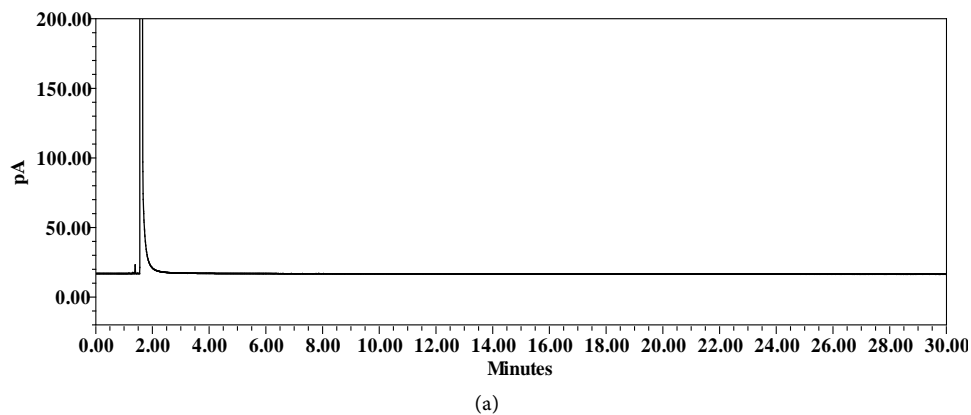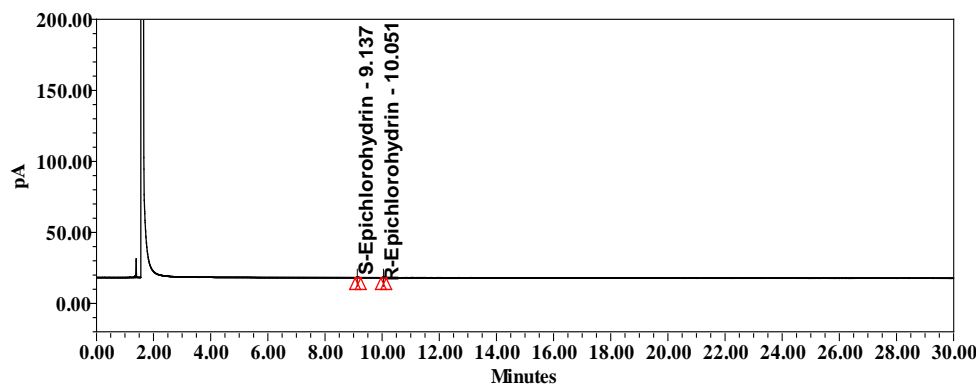
Figure 2. (a) Typical chromatograms of blank; (b) Typical chromatograms of system suitability; (c) Typical chromatograms of R-Epichlorohydrin sample; (d) Typical chromatograms of R-Epichlorohydrin sample spiked at specification level; (e) Typical chromatograms of LOQ; (f) R-Epichlorohydrin sample spiked at LOQ level.

FID detector) injecting six individual preparations of R-Epichlorohydrin sample spiked with 0.10% of S-Epichlorohydrin (0.10% of S-Epichlorohydrin isomer with respect to 20 μL/mL R-Epichlorohydrin). The RSD of peak area for S-Epichlorohydrin was calculated. The intermediate precision of the method was also evaluated using different analysts, different instruments and different columns and performing the analysis on three different days.

3.2. Accuracy

For determination of accuracy of method recovery study was carried out by analyzing the spiked samples. Known amount of S-Epichlorohydrin was spiked in triplicate at three different concentration levels of 0.01, 0.02 and 0.03 μL/mL (50%, 100% and 150% of the analyte concentration *i.e.* 20 μL/mL) to the drug product. The % recoveries for S-Epichlorohydrin was calculated based on mentioned in Equation (1).

$$\% \text{ Recovery of S} - \text{Epichlorohydrin} = \frac{\% \text{S} - \text{Epichlorohydrin Found}}{\% \text{ S} - \text{Epichlorohydrin added}} \times 100 \qquad (1)$$

3.3. Linearity

To establish linearity of the method was prepared by diluting stock solution to the required concentration. The solutions were prepared at seven concentration levels from LOQ to 150% of the specification level (LOQ, 0.025, 0.050, 0.075, 0.10, 0.125and 0.150%) with respect to the normal sample concentration (20 μL/mL). The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined.

3.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ for S-Epichlorohydrin were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting six (n = 6) individual preparations and calculating the RSD of the area of S-Epichlorohydrin.

3.5. Robustness

The robustness of an analytical procedure is a measure of its capability to remain unaltered by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

To determine the robustness of the method, the chromatographic conditions were deliberately changed and relative standard deviation of the S-Epichlorohydrin peak was evaluated. As the flow rate was 25 psi to study the effect of flow rate on relative standard deviation of the S-Epichlorohydrin peak, the flow rate was changed to 20 psi and 30 psi. The effects of the column oven temperature were studied at 45°C and 55°C instead of 50°C.

3.6. Solution Stability

The stability of S-Epichlorohydrin in R-Epichlorohydrin solution was determined by leaving test solutions of the sample and spiked solution in tightly capped volumetric flasks at room temperature for 24 hrs during which they were analysed at 12 hrs intervals.

4. Results

4.1. Method Development and Optimization

The main goal of method development was to achieve separation of S-Epichlorohydrin in R-Epichlorohydrin without derivatization. An understanding of the nature of the racemic Epichlorohydrin is the foremost prerequisite for successful method development in GC. Following were the stepwise strategies for the method development in our case.

4.1.1. Column Selection

The primary goal of column selection was to separate S-Epichlorohydrin and R-Epichlorohydrin from each other, which were used during the synthesis of Rivaroxaban. As part of method development screened various columns, namely Chiral GTA (30

meters × 0.25 mm I.D, 0.12 μm) and Chiralsil (30 meters × 0.25 mm I.D, 0.25 μm) were employed but no adequate separation was found with above columns. After careful screening of columns, it was observed that Gamaa-Dex-225 (30 meters × 0.25 mm I.D, 0.25 μm) column provides better resolution between S-Epichlorohydrin and R-Epichlorohydrin and it showed good system suitability parameters.

4.1.2. Flow Rate

As the flow rate increase, the viscosity of carrier gas decreased and velocity increased. Check the flow rate from 6 psi to 40 psi. At 6 psi, the retention time was very high and runtime is long, poor separation was observed at 40 psi. 25 psi was selected as finalized flow rate.

4.1.3. Selection of Diluent

Diluent selection study was conducted for the analysis of R-Epichlorohydrin and S-Epichlorohydrin. Four diluents had been tried N-methyl-2-pyrrolidone, Dimethyl formamide, Dimethyl imidazolidine and Dichloromethane. Dichloromethane was finalized as diluent because of no interference at the S-Epichlorohydrin and R-Epichlorohydrin peaks.

4.1.4. Injection Volume

The effect of injection volume on the quantification of the S-Epichlorohydrin and R-Epichlorohydrin were investigated by injecting volume between 0.5 μL to 2 μL of the standard solution. The results show that the peak widths of S-Epichlorohydrin and R-Epichlorohydrin were independent of injection volume within the tested range.

System suitability results are shown in Table 1 and Figures 3(a)-(c).

5. Method Validation

5.1. Precision

The % RSD for the content of S-Epichlorohydrin in the method precision was found to be less than 1.1. The % RSD for the content of S-Epichlorohydrin in the intermediate precision (Ruggedness) was found to be less than 2.2 (Table 2). The results confirmed the high precision of the developed GC method.

5.2. Limit of Detection and Quantification

The obtained limit of detection and limit of quantification, precision and accuracy at limit of quantification values are given in Table 2 and Figure 2(e), Figure 2(f).

Table 1. System suitability parameters.

Compound	RT (min)	RRT[a] (n = 6)	%RSD[b] (n = 6)[c]
S-Epichlorohydrin	9.169	0.85 ± 0.01	0.54
R-Epichlorohydrin	10.721	1.00 ± 0.00	

[a]Relative retention times (RRT) were calculated against the retention time (RT) of R-Epichlorohydrin. [b]Relative standard deviation. [c]Mean ± SD.
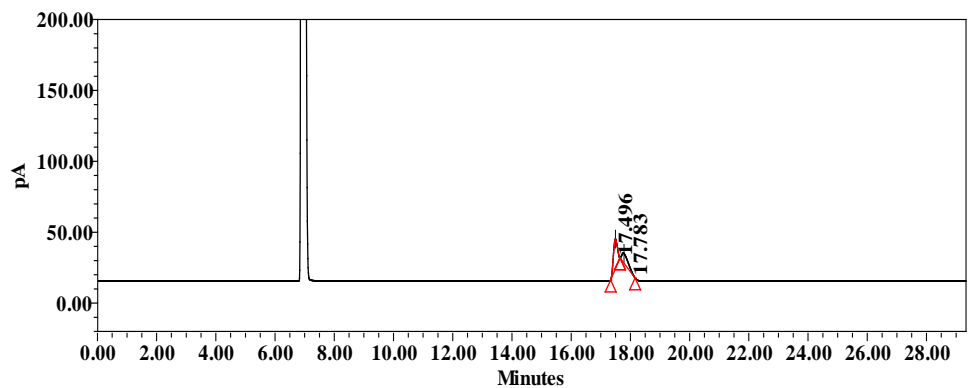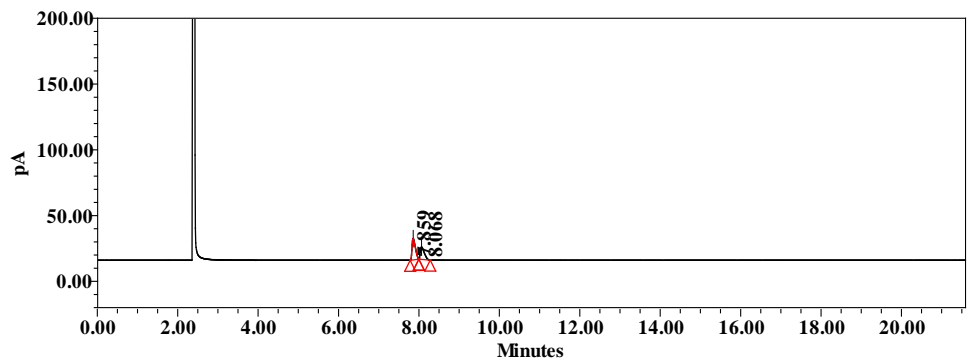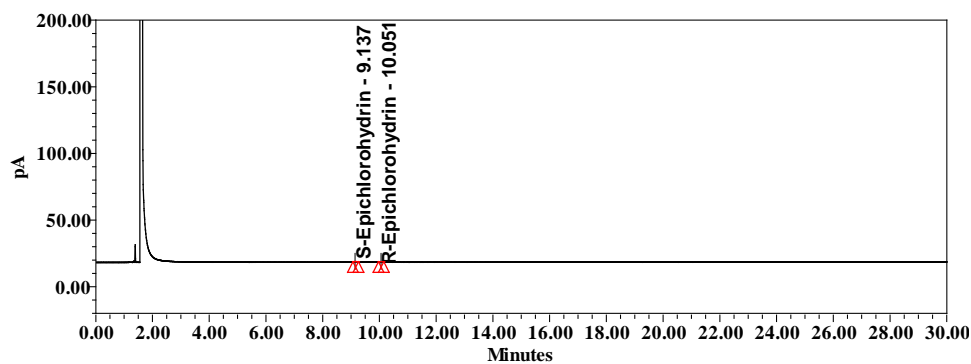
Figure 3. Method development chromatogram (a) method development chromatography conditions: chiralsil (30 m × 0.25 mm I.D, 0.25 μm) oven temperature 50°C for 30 minutes. Resolution between S-Epichlorohydrin and R-Epichlorohydrin was 0.9. Method development chromatogram (b) method development chromatography conditions: chiral gta (30 m × 0.25 mm I.D, 0.12 μm) oven temperature 50°C for 30 minutes. Resolution between S-Epichlorohydrin and R-Epichlorohydrin was 1.2 method development chromatogram (c) method development chromatography conditions: gamaa-dex-225 (30 meters × 0.25 mm I.D, 0.25 μm) oven temperature 50°C for 30 minutes. Resolution between S-Epichlorohydrin and R-Epichlorohydrin was 4.3.

5.3. Accuracy

Individual and average recoveries of three preparations and at three concentrations for S-Epichlorohydrin were within 100% ± 5% results shown in **Table 2** & **Table 3**.

Table 2. Summarized data of method validation.

Parameter	S-Epichlorohydrin
Linearity	
Correlation coefficient	0.9998
Slope	137034.3
Y-Intercept	0.0
%Y-Intercept	0.22
Accuracy (% Recovery)	
LOQ (n = 3)	98.2
50% (n = 3)	102.8
100% (n = 3)	101.5
150% (n = 3)	99.8
Precision (% RSD)	
LOQ (n = 6)	3.29
50% (n = 6)	1.98
100% (n = 6)	1.08
150% (n = 6)	0.98
Ruggedness; Different day and analyst (%RSD)	
100% (n = 6)	2.16
Robustness (%RSD)	
Actual flow 25 psi	0.80
Different flow 20 psi	1.34
Different flow 30 psi	1.09
Different Column Temperature 45°C	2.12
Different Column Temperature 55°C	1.98
Limit of Detection (Concentration in µg/mL)	0.001
Limit of Quantification (Concentration in µg/mL)	0.004

5.4. Linearity

The calibration curve was drawn by plotting the peak area against the concentration. The correlation coefficient (r^2) obtained was >0.9998. The % Y-intercept with respect to response at 100% level was >± 5%. The results for the Correlation coefficient (r^2) and % Y-intercept with respect to response at 100% level were shown in Table 4 & Table 5. The results demonstrate that an excellent correlation existed between the peak area and concentration of S-Epichlorohydrin.

5.5. Robustness

In all the deliberately varied chromatographic conditions, no effect on the Relative standard deviation of the S-Epichlorohydrin peak (Table 2) the method was more robust within the normal operating range, *i.e.*, column oven temperature, 50°C ± 5°C and flow rate, 25 ± 5 psi, demonstrating the robustness of the method results shown in Table 2.

Table 3. S-Epichlorohydrin accuracy and % RSD at 50%, 100% and 150% level.

Spike	S.No.	% of S-Epichlorohydrin in spiked sample	Avg S-Epichlorohydrin in sample	% of S-Epichlorohydrin found	% of S-Epichlorohydrin added	% Recovery	Avg. % Recovery
	Prep-1	0.1003	0.0500	0.0503	0.050	100.6	
	Prep-2	0.1021	0.0500	0.0521	0.050	104.2	102.8
	Prep-3	0.1018	0.0500	0.0518	0.050	103.6	
50%	AVG	0.10					
	SD	0.00					
	RSD	0.95					
	Prep-1	0.1525	0.0500	0.1025	0.100	102.5	
	Prep-2	0.1514	0.0500	0.1014	0.100	101.4	101.5
	Prep-3	0.1506	0.0500	0.1006	0.100	100.6	
100%	AVG	0.15					
	SD	0.00					
	RSD	0.63					
	Prep-1	0.1960	0.0500	0.1460	0.150	97.3	
	Prep-2	0.2001	0.0500	0.1501	0.150	100.1	99.8
	Prep-3	0.2032	0.0500	0.1532	0.150	102.1	
150%	AVG	0.20					
	SD	0.00					
	RSD	1.81					

Table 4. S-Epichlorohydrin linearity.

S.No	Conc	Actual concentration in mg/mL	S-Epichlorohydrin Area
1	LOQ	0.00000403	0.520
2	25%	0.00000586	0.870
3	50%	0.00001173	1.592
4	75%	0.00001759	2.426
5	100%	0.00002346	3.200
6	125%	0.00002932	4.050
7	150%	0.00003519	4.821
	Correlation	0.9998	
	Slope	137034.3	
	y-intercept	0.0	
	% y-intercept	0.22	

S-Epichlorohydrin Linearity
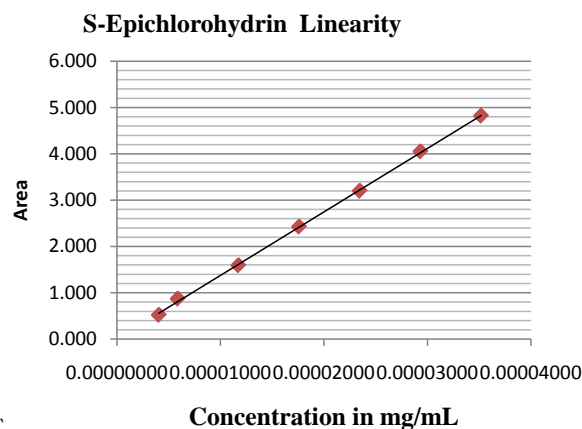
Table 5. S-Epichlorohydrin ANOVA.

SUMMARY OUTPUT				
Regression Statistics				
Multiple R	0.999782629			
R Square	0.999565306			
Adjusted R Square	0.999478367			
Standard Error	**0.036929457**			
Standard Error × 3	**0.11078837**			
Observations	7			

X Variable 1 Residual Plot

ANOVA

	df	SS	MS	F	Significance F
Regression	1	15.67989908	15.67989908	11497.34	0.00000000
Residual	5	0.006818924	0.001363785		
Total	6	15.686718			
	Coefficients	**Standard Error**	t Stat	P-value	Lower 95%
Intercept	0.007058657	0.02709363	0.260528295	0.804845	−0.062587736
X Variable 1	137034.2905	1277.999082	107.2256565	1.34E-09	133749.0893
	Upper 95%	Lower 95.0%	Upper 95.0%		
	0.07670505	−0.062587736	0.07670505		
	140319.4918	133749.0893	140319.4918		

RESIDUAL OUTPUT

Observation	Predicted Y	**Residuals**	Standard Residuals
1	0.559970752	−0.039970752	−1.1856596
2	0.810709957	0.059290043	1.758731197
3	1.614361258	−0.022361258	−0.663306009
4	2.418012558	0.007987442	0.236932931
5	3.221663858	−0.021663858	−0.642618921
6	4.025315158	0.024684842	0.732230897
7	4.828966459	−0.007966459	−0.236310495

5.6. Solution Stability

No significant change in the amounts of S-Epichlorohydrin was observed during solution stability experiments. The results from solution stability experiments confirmed that sample and spiked solutions were stable for up to 24 hrs results shown in Table 6.

6. Discussion

Based on the results, the successful separation of S-Epichlorohydrin and R-Epichlorohydrin from each other. All the validated parameters were found to be within limits. System suitability for 6 injections % RSD was found to be NMT 0.54%. Precision at LOQ, 100% and 150% were found to be NMT 3.29%, Accuracy at LOQ, 50% 100% and 150% were found to be 98.2% to 102.8%. Linearity was performed from LOQ to 150% and graph obtained was linear showing correlation coefficient >0.9998.

Table 6. S-Epichlorohydrin solution stability.

Sample "0" Hrs	Sample after 12 Hrs	Sample after 24 Hrs	Spiked "0" Hrs	Spiked after 12 Hrs	Spiked after 24 Hrs
% of Area	% of Area	% of Area	% of Area	% of Area	% of Area
0.0500	0.0502	0.0503	0.1525	0.1528	0.1530
Difference	0.0	0.0	Difference	0.0	0.0
% Difference	0.4	0.6	% Difference	0.2	0.3

7. Conclusion

A simple gas chromatographic method was developed and validated for the quantitative S-Epichlorohydrin in R-Epichlorohydrin for Rivaroxaban. S-Epichlorohydrin and R-Epichlorohydrin were well separated from each other, indicating that the developed GC method was specific. The method validation data showed satisfactory results for all tested method parameters. This simple GC method is precise, accurate, linear and rugged. Hence, it is proved that developed method can be used for routine testing in quality control laboratories for estimation S-Epichlorohydrin in R-Epichlorohydrin for Rivaroxaban. The method is user-friendly and robust to operate.

Acknowledgements

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