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METHOD DEVELOPMENT AND VALIDATION OF EPICHLOROHYDRIN CONTENT IN RANOLAZINE DRUG SUBSTANCE BY GC-MS/MS

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ABSTRACT

A GC-MS/MS method was developed for determination of Epichlorohydrin in Ranolazine drug substance using Rtx-624 column (30m X .32mm X 1.8µm) and a mobile phase of Helium gas with gradient GC oven temperature programming, at flow rate of 1.5ml/min with MS detector. The mass of Epichlorohydrin were found 57, 49 and 62 respectively. The retention time was found 11.4 minutes. The proposed method was validated for System suitability, Specificity, Linearity, LOD and LOQ determination, Recovery, Precision, and Range. All the parameters were found within the acceptable limits. The Linearity of Epichlorohydrin was in the range of 0.155µg/gm to 0.783µg/gm of specification limit. GC-MS/MS method was specific, accurate, precise and suitable for the analysis of Epichlorohydrin in Ranolazine drug substance.

KEYWORDS

Gas chromatography with mass spectrometry (GC-MS/MS), Genotoxic impurity, ICH guideline and Method validation.

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INTRODUCTON

Genotoxic impurities are undesirable chemicals, have no beneficial for health and are potentially harmful for direct DNA damage. Therefore, they need to be controlled in Active pharmaceutical ingredient and Drug products. Genotoxic impurities are those substances which impact DNA by means of transformations. Mutations can be chromosomal breaks, rearrangements, covalent binding or insertion into DNA during replication. Mutations may also occur indirectly by activating a cell to produce genotoxic substances. The focus of this study is on reactive substances they have a potential to directly

cause DNA damage when present low levels lading to mutations and there for potentially causing cancer. Because of this, it is important to identify genotoxic substances followed by monitoring and control at very low levels to ensure safety to the public.

The source of genotoxic impurities in pharmaceuticals (API and DP) can come from many places including starting materials, by products, reagents, intermediates, degradation products, ligands and catalysts, solvents or unwanted side reactions from the Active pharmaceutical ingredient synthetic process that get carried forward into the final product. In addition, the Active pharmaceutical ingredient itself can decompose to form genotoxic substances or they can form in the final product by reaction between excipients or containers and the active pharmaceutical ingredient.

The use of these substances within the synthetic procedure is logical as these compounds are reactive fragment that come together to form complex drug substances.

Impurity guidelines have mainly been developed by international Conference on Harmonization (ICH). ICH Q3A regulates impurities in new drug substances with thresholds for reporting, identifying, and qualifying impurities. ICH Q3B is the equivalent guideline for impurities in new drugs. ICH Q3C controls residual solvent, and is the first time the ICH applied substance specific limits. Depending on their potential risk to human health. At this time ICH Q3D is developed and included elements and limits for heavy metal impurities. At present the ICH guidelines for genotoxic impurity limits are not suitable. The genotoxins material considered unsafe at any level. The limit for a genotoxin with an understood toxicity can be calculated based upon the know PDE. The limit for the genotoxin without sufficient information must determine based upon TTC of 1.5µg/day.

METHOD DEVELOPMENT

Instrument, Chemicals and Reagents

The following reagents and chemicals were used during the evaluation studies.

Instrumentation

A Shimadzu Gas chromatography system (GC 201 Plus) with mass spectrometer (GCMS TQ8050) equipped with an auto sampler with Real time analysis software. Column was employed in the method was Restek Rtx-624 (30m X .32mm X 1.8µm). The flow rate selected was 1.5ml/min. All the weighing in the experiments was done with Mattler toledo electronic balance capable of measuring with an accuracy of 0.01mg.

Glassware

All the volumetric glassware used in the study was grade a quality Borosil.

Preparation of Diluent

Use 1-Methyl-2-pyrrolidone as diluent.

Preparation of Blank

Transfer 1.0mL of diluent into 20mL headspace vial. Close the vial with rubber septum and seal with aluminum crimp cap.

Preparation of Standard Solution

Accurately weigh and transfer about 95.0mg of Epichlorohydrin working/reference standard into a clean and dry 50.0mL of volumetric flask. Add diluent to dissolve the content and dilute to the volume with diluent.

Transfer 0.5mL of this solution into a clean and dry 50.0mL of volumetric flask and dilute to the volume with diluent. Further transfer 1.0mL of this solution into a clean and dry 50.0mL of volumetric flask and dilute to the volume with diluent. Label this solution as Standard Solution.

Transfer 1.0mL of standard solution into 20mL headspace vial. Close the vial with rubber septum and seal with aluminum crimp cap.

Preparation of Test Solution

Weigh accurately about 500.0mg of sample and transfer into 20mL headspace vial. Add 1.0mL of diluent. Close the vial with a rubber septum and seal with an aluminum crimp cap.

VALIDATION PARAMETERS

Specificity

The specificity is defined as the ability to assess and ensure that the impurities, degradation product and diluent do not affect the sample analyzed.

Inject the Blank (as Diluent), Standard solution, sample solution, and spike sample of impurities at

specification level. Check the interference at the retention time and mass of analyte.

No peak was observed in blank at the retention time of Epichlorohydrin peak. There is no interference observed in blank and other components presents in sample matrix with analyte peak. Hence method is specific. For details, refer Table No.1.

Limit of detection (LOD) and Limit of quantitation (LOQ)

It is the smallest amount or concentration of an analyte that can be estimated with acceptable reliability. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The limit of detection is determined by establishing the signal to noise ratio. Inject the blank and standard solutions at lower concentration and calculate the signal to noise ratio.

A signal-to-noise ratio between 3:1 estimating the detection limit.

The detection limit and quantitation limit for Epichlorohydrin in Ranolazine Drug substance 0.16mcg/g and 0.31mcg/g respectively. For details, refer Table No.2.

Precision at detection limit and Limit of quantitation

The Epichlorohydrin peak is detected reliably in six replicate injections at DL level. Hence obtained concentration can be considered DL level for Epichlorohydrin. The %RSD of area for peak Epichlorohydrin should be less than 33%.

%RSD of peak area of Epichlorohydrin in six replicate injection of LOD precision solution was found 3.1%. For details, refer Table No.3.

The Quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The limit of quantification is determined by establishing the signal to noise ratio. Inject the blank sample and the spiked sample at LOQ level in six replicates and calculate signal to noise ratio and the % RSD at LOQ level. The % RSD of peak area for six replicated injections of LOQ precision solution for Epichlorohydrin should not be more than 15.0%.

%RSD of peak area of Epichlorohydrin in six replicate injection of LOQ precision solution was found 1.6%. For details, refer Table No.4.

Precision

System precision

System precision was determined by injecting blank and six replicates of standard preparation. %RSD was calculated for Epichlorohydrin area response.

Prepared blank and standard solution as per description of analytical method.

Injected blank, standard solution, and checked the acceptance criteria for system suitability. For details, refer Table No.5.

Method Precision

Method precision was determined by analyzing six sample preparations as per the method representing a single batch.

Determined the results of these samples and evaluate the precision of the method by computing the %RSD results for Epichlorohydrin. The %RSD for Epichlorohydrin from six set of test preparation (above LOQ) should be NMT 20.0

Prepared blank and standard solution as per description of analytical method. %RSD for result for Epichlorohydrin of six sample is not applicable as it was found not detected hence it will not consider for evaluation. For details, refer Table No.6.

Since results of Epichlorohydrin was found not detected, then performed the spiked test repeatability by spiking Epichlorohydrin at specification level in the sample and injected in six replicates. Epichlorohydrin content was calculated. %RSD for result of Epichlorohydrin of six spiked sample was found 2.4%. For details, refer Table No.7.

Intermediate Precision

Intermediate precision was determined by analyzing six sample preparations as per the method representing a single batch by different analyst on different day. % RSD for Epichlorohydrin results were calculated.

Since results of Epichlorohydrin observed not detected in all test preparation during method precision study, then six individually test sample

spiked with Epichlorohydrin at specification level was analyzed in intermediate precision.

Prepared blank, standard solution and sample solution as per description of analytical method and injected. Results of intermediate precision refer Table No.8.

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the analyte by dilution of a standard stock solution using the proposed procedure. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods by calculation of a regression line. The correlation coefficient, y-intercept, slope of the regression line should be calculated.

The total number of seven concentration LOQ to 200% of specification levels considered and inject the duplicate injection of each concentration level to define a calibration graph. The acceptable value of the correlation coefficient (r2) should be more than 0.99 for Epichlorohydrin.

Correlation coefficient for the linearity curve of Epichlorohydrin in Ranolazine drug substance found >0.99. The method is found linear from LOQ to 200% of sample Concentration, for details, refer Table No.9.

Recovery

Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure.

Recovery assessed using a minimum of 3preparations over a minimum of 3 concentration levels (3 concentrations/3 replicates each level). % recovery was calculated for each level.

Acceptable limits for a recovery result during validation should be within the range of 70% - 130%. However, the lower recovery may be acceptable if the results are consistent (i.e. good precision).

Prepared blank, sample and standards solution as per methodology. Injected blank, sample and standards solution and checked the acceptance criteria for system suitability. Accuracy was carried out for Epichlorohydrinat QL level, 100% and 150% of specification level.% Accuracy for each level was found within acceptance criteria refer Table No.10.

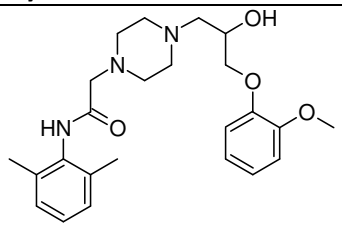
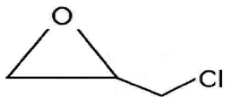
Range

Range of Epichlorohydrin content in Ranolazine drug substance are linear, precise, and accurate from LOQ to 150% of specification level.

DISCUSSION

A chromatographic method involves demonstrating specificity, which is the ability of the method to accurately measure the Epichlorohydrin response in the presence of all potential sample components. The chromatographic and mass spectroscopy parameters were fixed and GC-MS/MS system was studied for suitability of residual analysis. The developed method was performed for linearity, precision, Accuracy, specificity, range, LOD and LOQ.

Chemical Structure of Ranolazine and Epichlorohydrin

Ranolazine: **Chemical Name***: N-(2, 6-dimethylphenyl)-2-(4-(2-hydroxy-3-(2-methoxyphenoxy)propyl)piperazin-1-yl) acetamide* **Molecular weight***:* 427.54	
Epichlorohydrin **Chemical Name***:* (1-chloro-2, 3-epoxy propane) **Molecular weight***: 92.52g/mol* **Molecular Formula***: C_3H_5ClO*	

S.No	Name of the materials	Grade	Make
1	1-Methyl-2-pyrrolidone	GC-HS	Biosolve
2	Epichlorohydrin	GC	Sigma
3	Ranolazine	API	Mankind

Chromatographic Conditions for GC

S.No	Parameters	Description
1	Column	Rtx-624, (30.0m × 0.32 mm, 1.8 µm)
2	Carrier Gas	Helium
3	Flow control mode	Linear velocity
4	Linear velocity	44.2cm/sec
5	Split ratio	1:1
6	Flow rate	1.5mL/min
Oven program of GC		
7	Initial Temp	40^0C, hold for 5.0 min
8	Ramp 1	5^0C / min to 100^0C, hold for 0.00 min
9	Ramp 2	15^0C / min to 220^0C, hold for 10.0 min
10	Run time	35.0 minutes

MS Conditions

Ion Source temperature	220.0°C	
Interface temperature	220.0°C	
Solvent cut time	8.0 min	
Detector gain Voltage	Relative to the tuning result	
Detector gain	+ 0.30kV	
Acquisition Mode	SIM	
Start Time	9.00 minutes	
End Time	14.0 minutes	
Ion (m/z)	Target-Ion	57
	Reference-Ion	49
	Reference-Ion	62

Headspace Conditions (HS-20)

Oven temperature	80.0°C
Sample line temperature	90.0°C
Transfer line temperature	100.0°C
Shaking Level	5.0
Multi injection count	1 time
Equilibrating time	15.0 min
Pressurize time	2.0 min
Pressurized equilibrating time	0.50 min
Load time	0.50 min
Load equilibrating time	0.10 min
Injection time	0.50 min
Needle flush time	10.0 min
GC cycle time	45.0 min

Table No.1 Results of Specificity

S.No	Solution	Peak Name	RT(min)	Target Ion	Reference Ion-1	Reference Ion-2
1	Blank	Epichlorohydrin	ND	ND	ND	ND
2	Standard solution	Epichlorohydrin	11.4	57	49	62
3	Test solution	Epichlorohydrin	ND	ND	ND	ND
4	Spiked test solution	Epichlorohydrin	11.4	57	49	62

Table No.2 LOD and LOQ values

S.No	Compound	LOD Level (ppm)		LOQ Level (ppm)	
		Standard Conc.	w.r.t test	Standard Conc.	w.r.t test
1	Epichlorohydrin	0.078	0.16	0.155	0.31

Table No.3 Precision at LOD level

S.No	Compound	Area of Solvent						
		1	2	3	4	5	6	%RSD
1	Epichlorohydrin	21200	21915	22528	22034	22972	21282	3.1

Table No.4 Precision at LOQ level

S.No	Compound	Area of Solvent						
		1	2	3	4	5	6	%RSD
1	Epichlorohydrin	54187	55342	52938	53654	53191	54225	1.6

Table No.5 System Precision

S.No	Parameter	Result	Acceptance criteria
1	The % relative standard deviation of six replicate injections of standard solution for **Epichlorohydrin**	1.5	Should not be more than 15.0%.

Table No.6 Method Precision

S.No	Compound	Sample Result (in ppm)								
		1	2	3	4	5	6	Mean	SD	%RSD
1	Epichlorohydrin	ND	ND	ND	ND	ND	ND	NA	NA	NA

Table No.7 Method Precision at specification level

S.No	Compound	Sample Result (in ppm)								
		1	2	3	4	5	6	Mean	SD	%RSD
1	Epichlorohydrin	0.77	0.72	0.74	0.76	0.74	0.76	0.74	0.018	2.4

Table No.8 Intermediate Precision

S.No	Compound	Sample Result (in ppm)								
		1	2	3	4	5	6	Mean	SD	%RSD
1	Epichlorohydrin	0.67	0.69	0.69	0.72	0.72	0.67	0.69	0.022	3.20

Table No.9 Linearity

S.No	Linearity Conc. level	Epichlorohydrin	
		Conc. (ppm)	Mean area
1	LOQ level	0.1566	52669
2	50% level	0.1957	68655
2	75% level	0.2936	109306
4	100% level	0.3915	152482
5	125% level	0.4893	190811
6	150% level	0.5872	228369
7	200% level	0.7829	315241
8	Correlation coefficient		0.9998
9	Squared Correlation coefficient		0.9996
10	Slope		416639.4127
11	Y-Intercept		-12769.72154
12	Residual sum of square		20419086.45
13	**Linearity plot for Epichlorohydrin**		

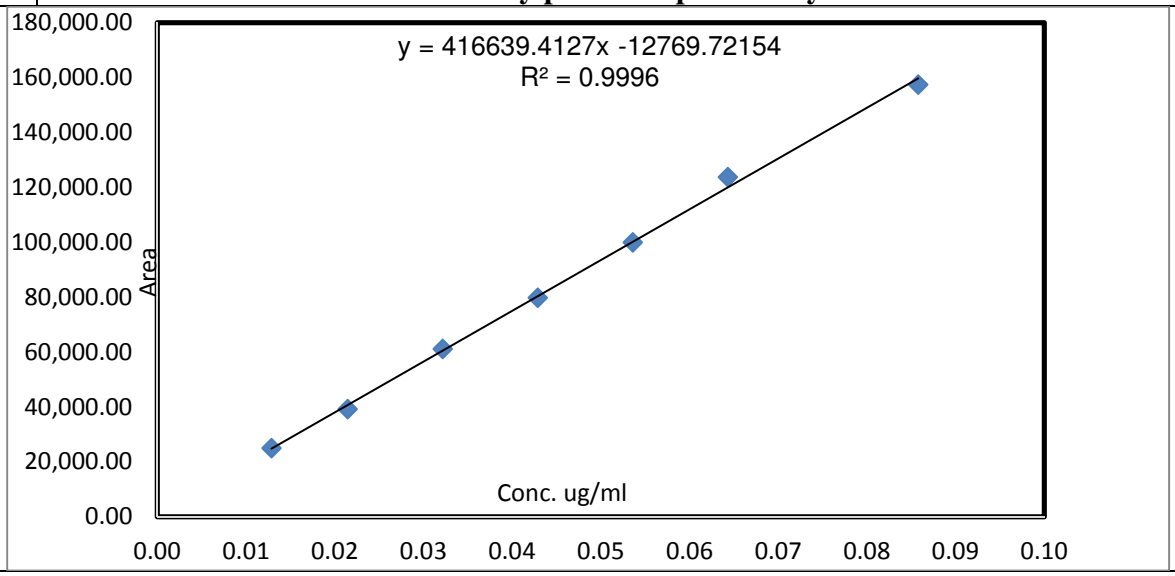


Table No.10 Accuracy (%Recovery)

S.No	Level (%)	Sample ID	Amount added (ppm w.r.t. Sample)	Amount recovered (ppm w.r.t. Sample)	Recovery (%)	Average Recovery (%)
1	Control	Injection-1	NA	NA	NA	NA
		Injection-2	NA	NA	NA	
		Injection-3	NA	NA	NA	
2	LOQ	Injection-1	0.307	0.2951	96.12	98.1
		Injection-2	0.307	0.3018	98.30	
		Injection-3	0.307	0.3063	99.77	
4	100	Injection-1	0.7676	0.9181	119.60	120.5
		Injection-2	0.7676	0.9419	122.70	
		Injection-3	0.7676	0.9156	119.28	
5	150	Injection-1	1.1514	1.3187	114.53	116.7
		Injection-2	1.1514	1.3427	116.61	
		Injection-3	1.1514	1.3688	118.88	
6	Recovery (Overall)			Mean	STDEV	%RSD
				111.75	10.542	9.4

Chromatograms of study

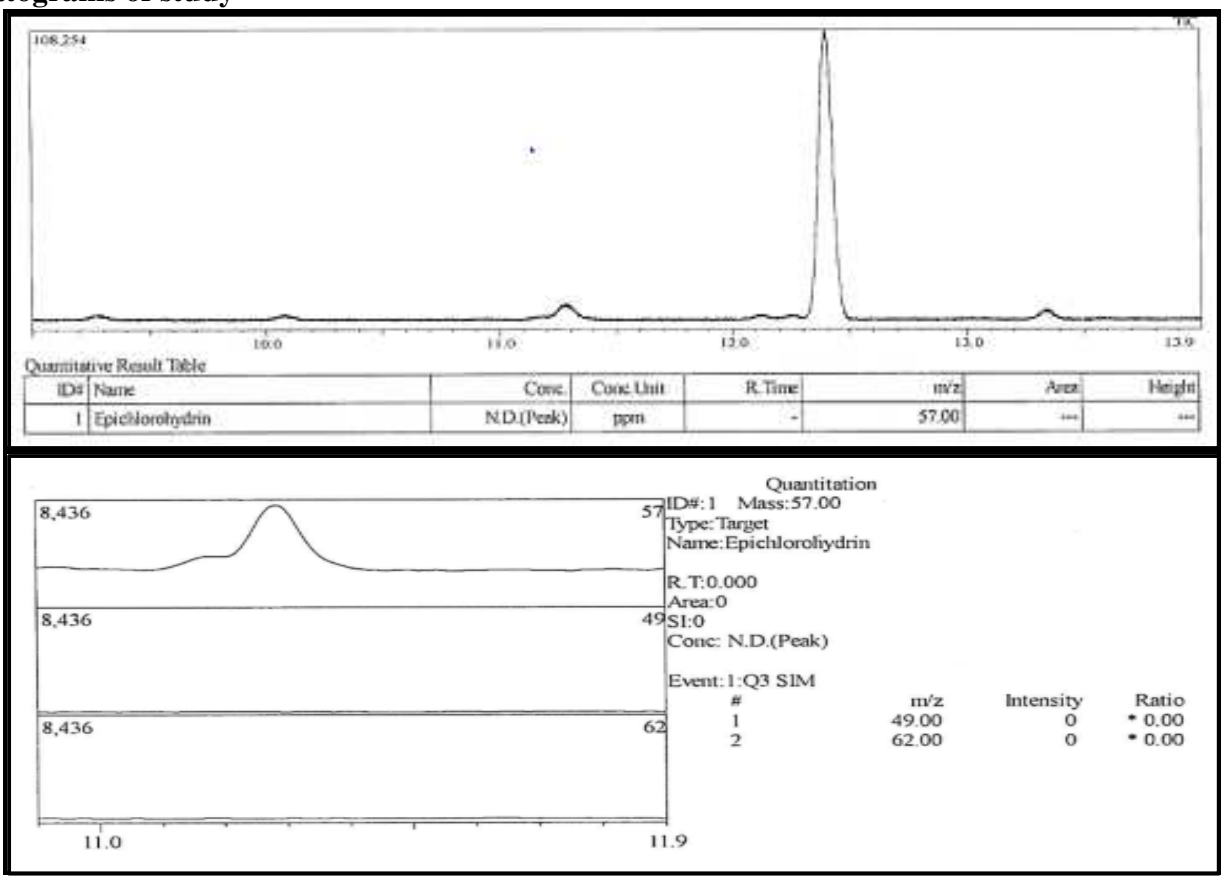


Figure No.1: Blank Chromatogram

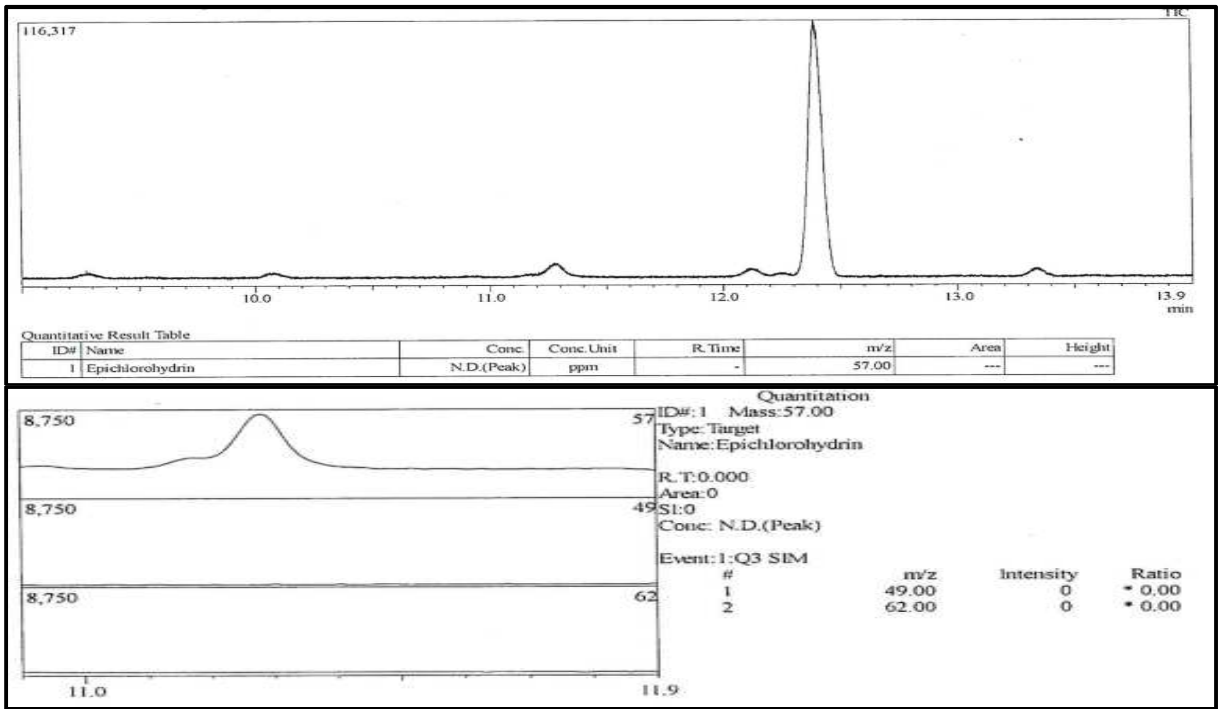


Figure No.2: Sample Chromatogram

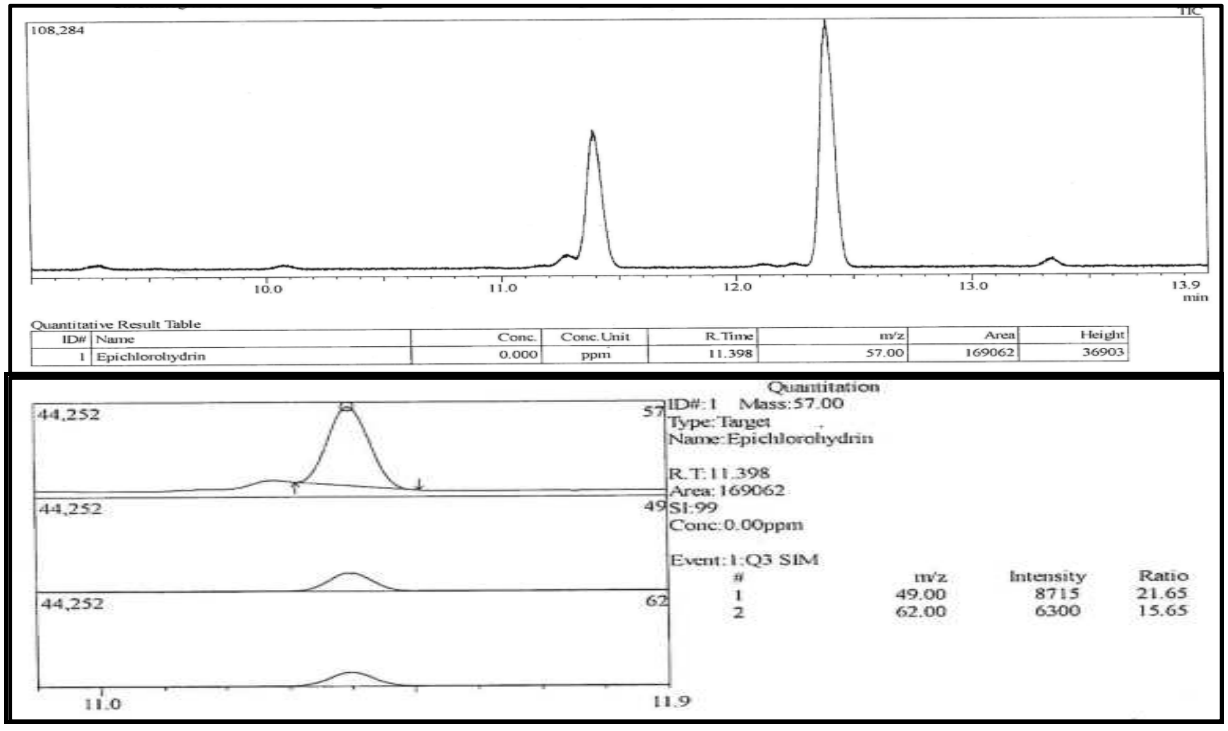


Figure No.3: Standard Chromatogram

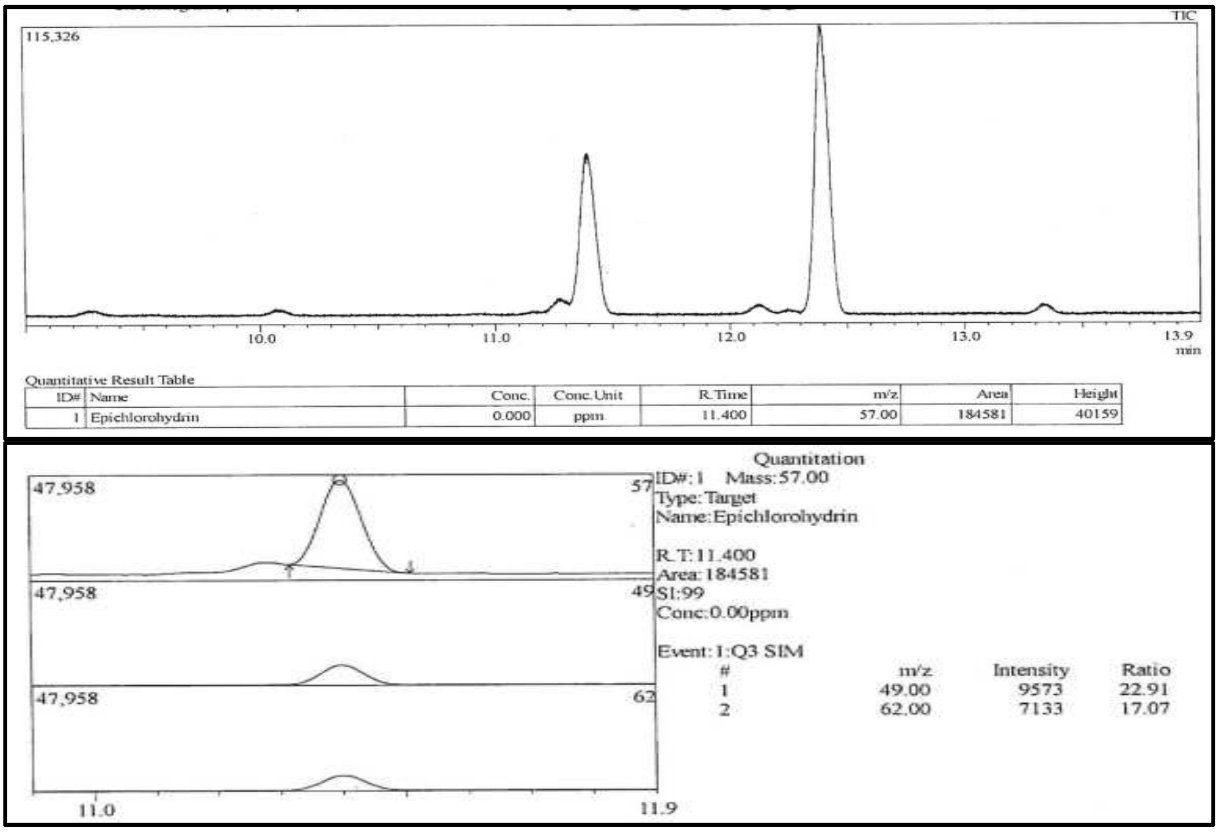


Figure No.4: Spike Sample Chromatogram

CONCLUSION

A simple and sensitive method for the determination Epichlorohydrin in Ranolazine drug substance by using GC-MS/MS was developed, validated and applied for the analysis of Ranolazine drug substance samples. The sample of Ranolazine drug substance was prepared with diluent. The present method was validated to secure the feasibility of the applied method for its application in day to day analysis. The LOQs achieved through this method were lower than the genotoxic impurities limit.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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