Available online on 15.06.2022 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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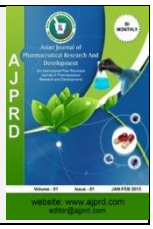

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Research Article

Development and validation of GCHSmethod used for trace level determination of carcinogenic Epichlorohydrin impurity in Rivaroxaban

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A B S T R A C T

A simple, sensitive, and selective Headspace-Gas chromatography (GCHS) method was developed and validated for the trace level determination of Epichlorohydrin, which is a probable carcinogenic compound and may be present as a process impurity in Rivaroxaban drug substances. Identification and separation of analytes were achieved on a DB-Wax column having dimensions of 30 m in length and a 0.53 mm internal diameter with a 1.0 µm stationary film thickness. Nitrogen is used as a carrier gas with of flow rate of 6.0 mL/min. The run time of the method is 25 minutes. The gradient column oven-temperature programming method was used for analyte elution. The developed method was validated using ICH guidelines and found a linear in the range between 9.64 ppm and 80.31 ppm. The correlation coefficient and y-intercept observed for the linearity curve are 0.9993 and 1.75%, respectively. The limit of detection and quantitation were 3.03 ppm and 9.19 ppm, respectively. Recovery was observed for Epichlorohydrin between 94.66 % and 99.22 %. The RSD for system precision, precision at LOQ, and repeatability was less than 5.0 %. The developed method can be successfully applied to determine Epichlorohydrin in Rivaroxaban drug substances up to a very low trace level concentration. The method was found to be specific, selective, linear, precise, and accurate.

Keywords: Rivaroxaban, genotoxic, Epichlorohydrin, impurity, development, and validation

A R T I C L E I N F O: Received; 19 April 2022; Review Complete; 01 May 2022 Accepted; 28 May 2022 Available online 15 June. 2022

Cite this article as:

Sunanna L, Manoharan A, Evaluation of Anti Microbial Activity of Classical Siddha Medicine Seenthil Choornam, Asian Journal of Pharmaceutical Research and Development. 2022; 10(3):30-33.

DOI: http://dx.doi.org/10.22270/ajprd.v10i31123

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INTRODUCTION

Rivaroxaban chemically known as 5-chloro-N-[[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide (Figure-1). Rivaroxaban is a factor Xa inhibitor of class anticoagulants, antithrombotic & cardiovascular drugs used to treat deep vein thrombosis, pulmonary embolism & reduce the risk of forming a blood clot in the legs and lungs [1-2]. 2-(Chloromethyl)oxirane or Epichlorohydrin is a versatile precursor in the synthesis of many organic compounds and belongs to a class of epoxide that is probably carcinogenic to humans and classified as Group 2A by the International Agency for Research on Cancer (IARC)[3]. Epichlorohydrin is a process impurity that may be formed during the synthesis of Rivaroxaban. An established risk assessment practice such as those used by international regulatory bodies may be applied either to calculate acceptable intakes or to use already existing values published by regulatory authorities for carcinogenic impurities. For epichlorohydrin acceptable intake of 3 µg/day is published in ICH M7(R2): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk 2nd Addendum[4-5].

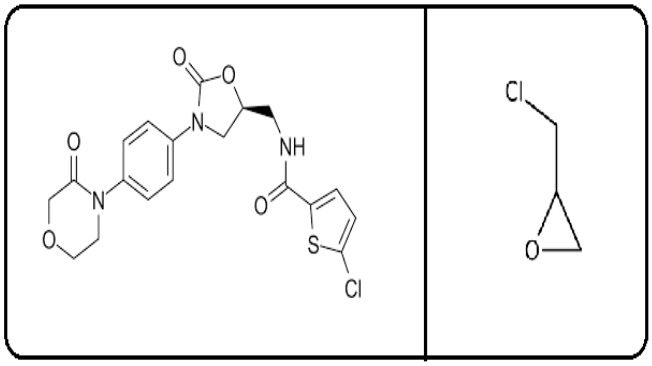

Figure1: Structure of Rivaroxaban and Epichlorohydrin

Hence, in order to meet the regulatory requirements, it is essential to develop a highly sensitive analytical method that can identify and determine Epichlorohydrin in Rivaroxaban upto the trace level. Considering the maximum daily dose of 30 mg/day for Rivaroxaban, the limit for Epichlorohydrin comes out to be 100 ppm. Hence, for this study, the limit for Epichlorohydrin is considered 50 ppm.

EXPERIMENTAL

Material& Chemicals

Rivaroxaban bulk drug sample was provided by the chemical research and development department (CRD) of Indoco Research Centre, Navi Mumbai. Epichlorohydrin was purchased from TCI chemicals whereas GC grade Dimethylsulfoxide was purchased from Qualigens. Headspace GCHS vials of 22 mL capacity of Perkin make having PTFE septa and crimp caps were used for the analysis. Volumetric flask and pipettes used for the analysis were of Class-A standard and Borosil make.

Instrumentation

Perkin Elmer gas chromatograph with headspace (Clarus) autosampler (Turbo matrix) along with flame ionization detector was used for the analysis, whereas the analytical balance of Sartorius (Germany) was used for weighing the materials.

Methodology

Chromatographic condition

Various GC columns were used for development, good separation and peak shape was achieved on DB-Wax GC capillary column having 100% Polyethylene glycol (PEG) stationary phase (Make-Agilent) Column dimension was 30 m in length and 0.53 mm of internal diameter and coated with the stationary phase of 1.0 µ m film thickness. Nitrogen (99.999%, purity) was used as the carrier gas with a constant flow rate of 6.0 mL/min. Details of other optimized gas chromatographic and headspace parameters are given in Table-1 and Table-2 respectively. For suitability of a system, tailing factor kept was not more than 2.0, theoretical plate not less than 5000 and % RSD of not more than 5.0% was kept for peak area of Epichlorohydrin for six standard solutions replicate injections.

Table1: Optimized gas chromatographic conditions

Detector	Flame Ionization Detector
Oven temperature	Initial 40°C, hold for 4.0 minutes
	Increase @ 10°C per minute to 200°C
	Hold at 200°C for 5.0 minutes
Injector Temperature	230°C
Detector Temperature	150°C
Attenuation	-6
Range	1
Split Ratio	1:1
Run Time	25 minutes

Table 2: Headspace conditions

Oven equilibration temperature	100°C
Needle temperature	110°C
Transfer line temperature	120°C
Thermostat time	15.0 minutes
Pressurization time	3.0 minutes
Withdrawal time	0.5 minutes
Injection time	0.5 minutes
GC cycle time	40.0 minutes

Preparation of solutions

Diluent: Dimethyl sulfoxide

Blank

Transfer 1.0 mL of diluent into a headspace vial and seal the vial immediately with an aluminum cap containing PTFE/silicon septa using a crimper.

Standard stock solution

Transfer about 0.05 g of Epichlorohydrin into a 100 mL volumetric flask containing about 20 mL of diluent, mix well and make upto the mark with diluent.

Standard solution

Transfer about 1.0 mL of Standard stock solution into a 100 mL volumetric flask and make upto the mark with diluent. Transfer about 1.0 mL of this solution into six headspace vials separately and seal the vials immediately with an aluminum cap containing PTFE/silicon septa using a crimper.

Test solution

Transfer about 0.1 g of Rivaroxaban sample into the headspace vial, add 1.0 mL of diluent and seal the vial immediately with an aluminum cap containing PTFE/Silicon septa using a crimper.

Procedure and calculation

Conditioned the column at 200°C and then equilibrated at 40°C. Injected blank solutions, six standard solutions and the test solution in duplicate. Recorded the peak area of each solvent and calculated solvent content in Neostigmine Methylsulfate by the formula below

Epichlorohydrin (ppm) = (AT/AS) x (WS/WT) x P

Where AT is the area of solvent in the test sample, AS is the average area of Epichlorohydrin in the standard solution, WS is the weight of Epichlorohydrin taken for standard solution preparation (g) and WT is the weight of the Rivaroxaban test sample (g).

Analytical method validation

The analytical method validation work is conducted according to the International Conference on Harmonization (ICH) guidelines. The parameter with which the analytical method is validated is Specificity, Limit of detection, Limit of quantitation, Linearity, Accuracy and Precision [6-7].

Specificity

The capability of the method to measure the analyte peak (solvent) response in the presence of other components is termed specificity. For this, blank, standard, test sample, and spiked test sample solutions were injected and observed in the chromatogram for any interference from blank and test sample peaks at a retention time of Epichlorohydrin peak. It was observed that there was no interference at a retention time of Epichlorohydrin peak. (Figure-2). Also, the retention time of the Epichlorohydrin peaks for standard and the spiked test sample were matching (Table-3).

Table 3: Retention time of Epichlorohydrin peak

Sample ID	Retention time (min)
Standard solution	9.752
Test solution	9.753
Spiked Test solution	9.766
Blank	No peak

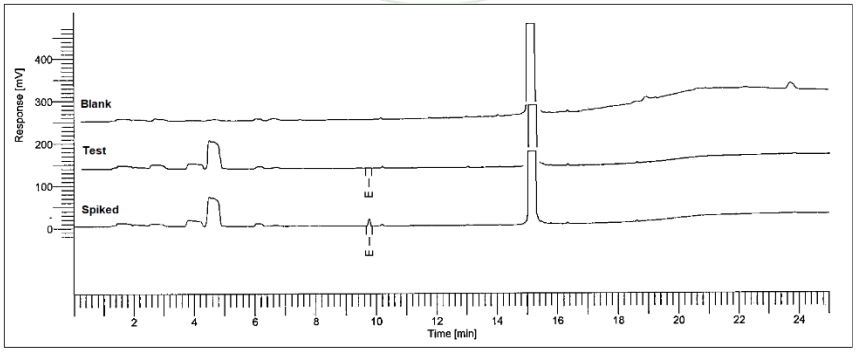

Figure 2: Chromatograms overlay

Limit of detection and quantitation

A Series of standard solutions of Epichlorohydrin was prepared in concentrations ranging from 50% to 150% of the target concentration (50 ppm w.r.t. sample). Limit of detection (LOD) and Limit of quantitation (LOQ) was calculated based on a residual standard deviation of the regression line and slope. The limit of detection obtained was 3.03 ppm and the Limit of quantitation was 9.19 ppm.

Linearity

A Series of linearity solutions of Epichlorohydrinwas prepared from LOQ to 150% of the target concentration (50 ppm w.r.t. sample). Linearity curves were drawn by plotting the peak area of Epichlorohydrinagainst the concentration of linearity solution (Fig.4). The regression coefficient observed was 0.9993 and % y-intercept 1.75.

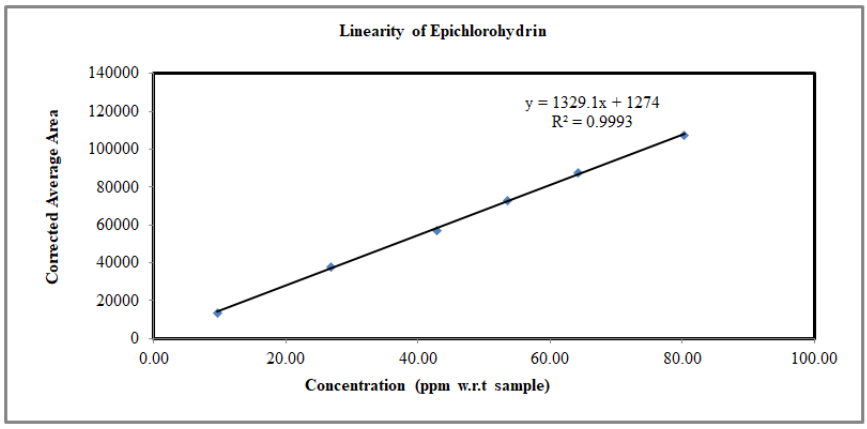

Figure-3: Linearity graph of Epichlorohydrin

Precision

System precision was carried out by analysing six standard solutions of Epichlorohydrin at the limit level concentration (50 ppm). The relative standard deviation for the peak area of Epichlorohydrinwas calculated and found to be 2.38 %. Precision at LOQ solution was prepared at LOQ concentration (9.64 ppm) of Epichlorohydrinand injected six times. The relative standard deviation for the peak area for Epichlorohydrinobtained was 2.21%. For repeatability, six solutions were prepared by spiking the Epichlorohydrin in the test sample at a limit level concentration (50 ppm). The relative standard deviation observed for spiked Epichlorohydrincontent in repeatability was 3.13 %.

Accuracy

The accuracy of the method was established by performing the recovery studies of Epichlorohydrin, for which it was spiked at LOQ, 100% and 150% in the Rivaroxaban test sample in triplicate and analyzed for its recovery. Recovery for Epichlorohydrin obtained was between 94.66% and 99.22%.

CONCLUSION

The developed Headspace gas chromatographic (GCHS) method is simple and does not require derivatization or highly sophisticated mass instruments. This method can be successfully applied for the quantitative determination of Epichlorohydrin in Rivaroxaban bulk drug samples on a manufacturing level in the pharmaceutical industry. The method is validated and found to be specific, linear, accurate, and precise. Acceptable data for all method validation parameters tested and found to be satisfactory.

ACKNOWLEDGEMENT

The author wishes to thank the management of Indoco Remedies Limited for supporting this work by providing the samples of Rivaroxaban and Epichlorohydrin standards required for this research.

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