IJPSR (2020), Volume 11, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 May 2019; received in revised form, 09 September 2019; accepted, 13 November 2019; published 01 March 2020

DEVELOPMENT AND VALIDATION OF GAS CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF GENOTOXIC IMPURITY, EPICHLOROHYDRIN, IN LINEZOLID DRUG

Duggirala Parvatha Venkata Vardhani Devi * [1], Kapavarapu Maruthi Venkata Narayanarao [1], Pulipaka Shyamala [2], Rallabhandi Murali Krishna [3] and Komali Siva Prasad [1]

GVK Biosciences [1], Pvt. Ltd., Hyderabad - 500076, Telangana, India.
Department of Physical Chemistry [2], Department of Physical and Nuclear Chemistry and Department of Chemical Oceanography [3], Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India.

Keywords:

Epichlorohydrin, Genotoxic impurity, Linezolid, Headspace, Gas chromatography, Flame ionization, Monitoring

Correspondence to Author:
Duggirala Parvatha Venkata Vardhani Devi

GVK Biosciences, Pvt. Ltd., Hyderabad - 500076, Telangana, India.

E-mail: dhanimaruthi@gmail.com

ABSTRACT: A sensitive and robust gas chromatography with a flame ionization detector method was developed for the quantification of epichlorohydrin in the linezolid drug substance and validated using guidelines as mentioned in ICH Q2 (R1). Epichlorohydrin was separated on CP-Volamine stationary phase (length 60 m, diameter 0.32 mm, particle size 5 µ) in linear thermal programming using dichloromethane as a diluent, at a constant flow rate of 2 ml/min. Column oven temperature maintained initiated at 90 ºC and raised to temperature 230 ºC with run time 25 min. Epichlorohydrin showed linearity from 5.12 ppm to 30 ppm concentration range. The method has satisfactory precision and accuracy. Limit of detection and limit of quantification were found as 2.03 ppm and 5.12 ppm, respectively. The epichlorohydrin peak has been well resolved and its specificity has been demonstrated. The method developed and validated for estimating epichlorohydrin in linezolid drug molecule is easy and simple to adopt in any pharmaceutical laboratory.

INTRODUCTION: Linezolid, called chemically as N- (((S)- 3- (3- Fluoro- 4-morpholinophenyl)-2-oxo-5-oxazolidinyl)methyl)acetamide **Fig. 1**, is a synthetic antibiotic of a new antibiotic class called oxazolidinone [1]. Linezolid is used in medication of multi-resistant bacteria, which include *Streptococcus* and *Staphylococcus aureus* resistant to methicillin [2, 3]. By binding to specific sites in the bacterial ribosome, linezolid preferentially inhibits protein synthesis in bacteria through prohibiting functional 70S initiation complex formation.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(3).1284-89

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(3).1284-89

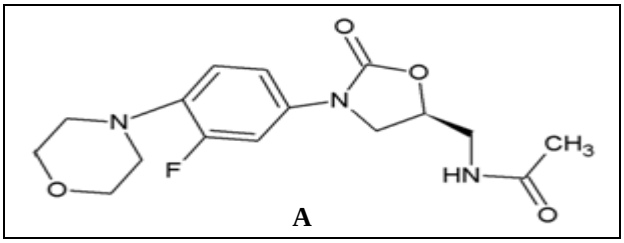

FIG. 1: STRUCTURES OF A. LINEZOLID AND B. EPICHLOROHYDRIN

Epichlorohydrin called chemically as 2-(chloro-methyl) oxirane **Fig. 1**, is an agile precursor in the manufacturing of so many organic molecules like pharmaceutical products, glycerol, epoxy resins, ion exchange resins, elastomers, water-treatment resins, surfactants, plasticizers, adhesives and

lubricants [4]. Epichlorohydrin is a molecule of epoxide class of compounds. Epichlorohydrin is used in the chemical process of linezolid synthesis [5]. Prolonged oral intake and through inhalation of elevated epichlorohydrin levels may cause problems higher risk of stomach cancer and lung cancer, respectively [6]. Due to its recognized genotoxicity and carcinogenicity, the appearance of residual epichlorohydrin in linezolid drug substance should be regulated according to EMA (European Medicines Agency) [7], ICH (International Conference on Harmonization) [8-10] and FDA (Food and Drug Administration) [11]. For pharmaceutical substances, the acceptable exposable risk for genotoxic impurity is 1.5 µg per day [12, 13]. Based on the above facts, the detection and monitoring of epichlorohydrin levels require a sensitive, accurate and robust analytical method.

There are few articles available for epichlorohydrin quantification in air [14], water [15-17], sewage sample [17] and active pharma ingredient - develamer hydrochloride and milnacipran hydrochloride [18, 19]. All the methods published are based on a strategy of gas chromatography coupled with a different detector system. No method has been documented to date to quantify epichlorohydrin in linezolid drug substances. This prompted us to develop of new gas chromatography with flame ionization detection method for determining epichlorohydrin in linezolid. The proposed gas chromatography with flame ionization detection method was validated using the guidelines of ICH and USP [20, 21].

MATERIALS AND METHODS:

Chemicals: Linezolid and epichlorohydrin were obtained from GVK Biosciences Private Limited, (Hyderabad, India). Dichloromethane was obtained from Merck (Mumbai, India).

Instrumentation: The entire experiments were executed on Agilent 6890 N model gas chromatography fitted with Headspace G1888 N, flame ionization detector and Empower software. Gas chromatography CP-Volamine, length 60 m x identification 0.32 mm, film thickness 5.0 µm was used.

Gas Chromatography Conditions: The specified conditions used to detect and monitor epichlorohydrin are given below:

Injection volume	:	4 µl		
Injector temperature	:	200 ºC		
Detector temperature	:	260 ºC		
Carrier gas	:	Nitrogen		
Split ratio	:	1:1		
Column flow	:	2.0 ml/min (constant flow)		
Oven temperature program	:	Ramp	Temp.	Hold Time
		0	90	5
		8	125	2
		10	230	3.11
Run time	:	25 min		

Standard Solutions of Epichlorohydrin: Epichlorohydrin stock solution (1000 ppm) was made by transferring 25 mg of epichlorohydrin accurately into a 25 ml volumetric flask with 5 ml of dichloromethane. Mix to dissolve completely and dilute to volume with dichloromethane.

For linearity studies, epichlorohydrin stock solution (1000 pm) was exactly diluted in 10 ml volumetric flasks with dichloromethane to get the concentration of 5.12 ppm, 10 ppm, 16 ppm, 20 ppm, 24 ppm, and 30 ppm.

For accuracy, precision and robustness studies, an appropriate volume of epichlorohydrin stock solution (1000 pm) was exactly diluted in 10 ml volumetric flasks with dichloromethane to get a concentration of 20.8 ppm.

Test Sample Solution: Dissolve correctly 1000 mg of linezolid in 5 ml of dichloromethane and cyclo mix for 2 min. Finally, dilute to volume with dichloromethane in a 10 ml volumetric flask (concentration of linezolid 100 mg/ml).

Procedure: Set up the gas chromatography system to the above chromatographic conditions. Allow the column to equilibration for one hour. Inject 4 µl of sample solution into the gas chromatography system. Determine the peak area of epichlorohydrin. Then calculate the concentration of epichlorohydrin using the below formula.

Average area of solvent peak in sample – Area of blank peak / Average area of solvent from standard – Area of blank peak × Concentration of standard in mg/ml × 10^6 / Concentration of sample in mg/ml

RESULTS AND DISCUSSION:

Optimization of Conditions: Dichloromethane was selected as a diluent as it did not show interference peak with epichlorohydrin peak. Gas chromatography column CP-Volamine (length 60 m × identification 0.32 mm, film thickness 5.0 µm) yielded a good peak shape. Nitrogen was used as carrier gas to get a steady and constant baseline. Column flow rate, split ratio and injection volume were set at 2 ml/min, 1:1 and 4 µl, respectively as these set values gave adequate sensitivity and excellent precision.

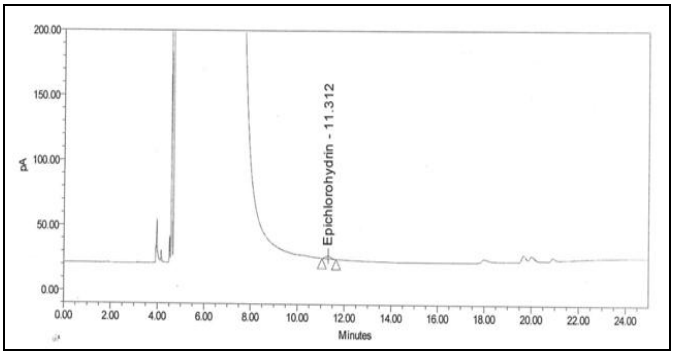

FIG. 2: CHROMATOGRAM OF EPICHLOROHYDRIN WITH OPTIMIZED GAS CHROMATOGRAPHY CONDITIONS

The temperatures at injector and detector were set at 200 ºC and 260 ºC. Using the above stated optimized conditions, epichlorohydrin was eluted from the column at a retention time of 11.312 min **Fig. 2**.

Validation: The proposed gas chromatography with flame ionization detection method was validated using the guidelines of ICH and USP [20, 21].

Specificity: To evaluate the method's specificity, dichloromethane blank, epichlorohydrin standard solution (20.8 ppm), linezolid sample (100 mg/ml) and epichlorohydrin spiked linezolid sample (20 ppm) preparations were subjected to gas chromatography with flame ionization detection. Chromatograms were collected.

No interference was found at the retention of epichlorohydrin (11.312 min) in the chromatograms of dichloromethane blank, linezolid sample and epichlorohydrin spiked linezolid sample as illustrated in **Fig. 3A-3D**. The results demonstrated specificity.

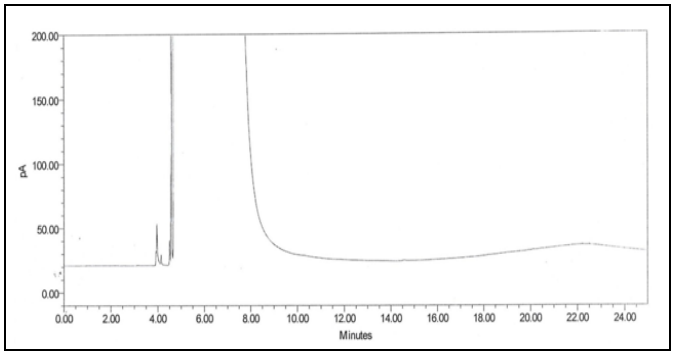

FIG. 3A: DICHLOROMETHANE BLANK CHROMATOGRAM

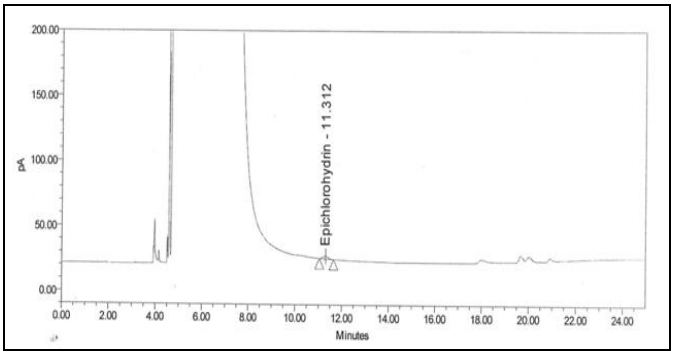

FIG. 3B: EPICHLOROHYDRIN STANDARD (20 ppm) CHROMATOGRAM

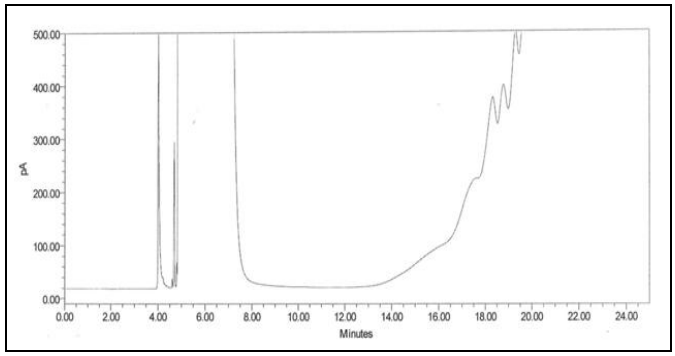

FIG. 3C: LINEZOLID SAMPLE (100 mg/ml) CHROMATOGRAM

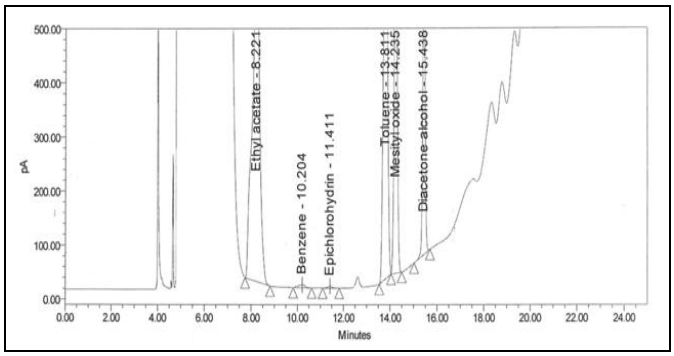

FIG. 3D: EPICHLOROHYDRIN (20 ppm) SPIKED LINEZOLID SAMPLE

Precision: Precision is the degree of repeatability under standard conditions for an analytical method. The method's precision was evaluated by calculating the relative standard deviation of six

replicate peak area determinations by injecting freshly prepared 20 ppm solutions of epichlorohydrin separately on the same day. The % RSD value was 1.18%. The low % RSD values via peak areas confirm the method's good precision.

TABLE 1: PRECISION STUDY RESULTS FOR EPICHLOROHYDRIN

Injection no.	Epichlorohydrin peak area
1	65.35
2	63.86
3	63.31
4	64.77
5	64.76
6	64.98
Average value	64.5
% RSD	1.18

Limits of Detection (LOD) and Quantitation (LOQ): Standard epichlorohydrin solutions were separately injected. The detection limit and quantification limit was then determined at the lowest concentration with a signal to noise proportion of 3 and 10, respectively, under the provided experimental conditions. The limits of detection and quantification values for epichlorohydrin were found as 2.03 ppm and 5.12 ppm, respectively. The values show the satisfactory sensitivity of the method for detecting and monitoring epichlorohydrin. Epichlorohydrin chromatograms at LOD and LOQ concentrations are illustrated in **Fig. 4A** and **4B**.

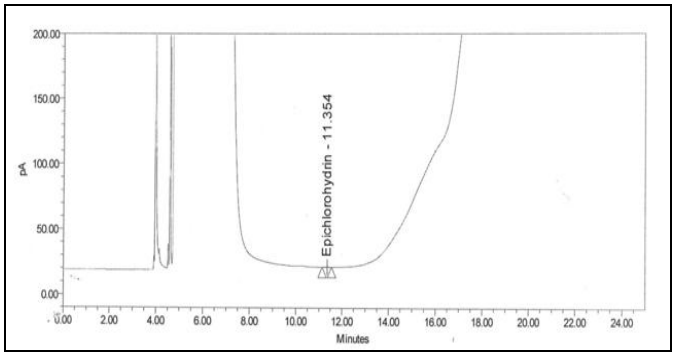

FIG. 4A: EPICHLOROHYDRIN CHROMATOGRAM AT LOD CONCENTRATION (2.03 ppm)

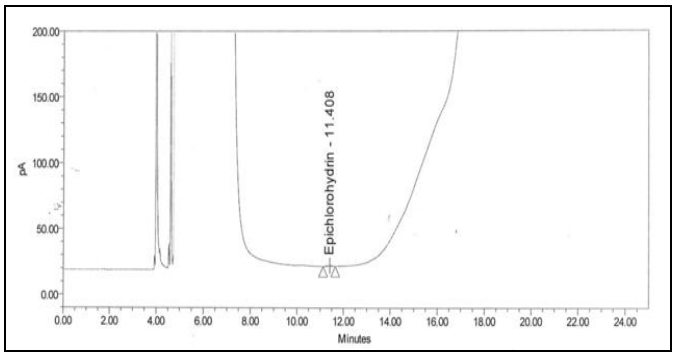

FIG. 4B: EPICHLOROHYDRIN CHROMATOGRAM AT LOQ CONCENTRATION (5.12 ppm)

Linearity: Linearity was evaluated by series of injections of six standards with concentrations ranging from LOQ to 150% (5.12 ppm, 10 ppm, 16 ppm, 20 ppm, 24 ppm, and 30 ppm) of the expected concentration range. The peak area of each concentration was determined using the described gas chromatography conditions. The response was proportionate to epichlorohydrin concentration. The calibration curve across the range of 5.12 to 31.3 ppm was drawn between peak areas versus epichlorohydrin concentration. The slope, intercept and correlation coefficient values were derived from linear least square regression treatment **Table 2**. The method's good linearity was proven by the correlation coefficient *viz.* 0.9986 for epichlorohydrin.

Accuracy: Method's accuracy was evaluated by standard method of addition. A known amount of epichlorohydrin spiked at four specification levels (LOQ, 50%, 100%, and 150%) to the linezolid sample solution. The spiked solutions were analyzed in triplicate by described gas chromatography conditions. The recovery must be more than or equal to 80% and should not be more than 120%. The recovery of epichlorohydrin at four specification levels was assessed **Table 3**. The recovery data show method's accuracy.

Robustness: Method's robustness was evaluated by making minor but intentional variations in column, injector, and detector temperatures. The effect of variations has been studied on the peak area of epichlorohydrin. The results are shown in **Table 4**. The percentage of RSD values of the peak area found to be inside the acceptance criteria in all varied conditions.

TABLE 2: LINEARITY AND LEAST SQUARE REGRESSION TREATMENT RESULTS FOR EPICHLOROHYDRIN

Level (%)	Concentration (ppm)	Area of peak
LOQ	5.12	16.6
50	10	31.3
80	16	54.1
100	20	66.9
120	24	78.5
150	30	103.9
Correlation coefficient		0.9986
Slope		3.476
Y-Intercept		-2.3504

TABLE 3: ACCURACY RESULTS OF EPICHLOROHYDRIN

S. no.	Theoretical concentration (ppm)	Found concentration (ppm)	Recovery (%)	Average (%)
LOQ level				
1	5.12	4.40	85.94	87.89
2	5.12	4.57	89.26	
3	5.12	4.53	88.48	
50% accuracy				
1	10	9.33	93.27	
2	10	8.37	83.65	90.06
3	10	9.33	93.27	
100% accuracy				
1	20	20.87	104.33	
2	20	17.60	87.98	95.51
3	20	18.85	94.23	
150% accuracy				
1	30	28.47	94.89	98.39
2	30	32.68	108.95	
3	30	27.40	91.35	

TABLE 4: ROBUSTNESS RESULTS OF EPICHLOROHYDRIN

Injection no.	As per the method	Temperature (ºC) at					
		Column oven		Injector		Detector	
		95	85	195	205	265	255
1	61.75	58.21	80.90	63.42	74.34	60.52	74.34
2	62.09	58.32	81.12	63.63	76.32	60.91	76.32
3	62.08	59.14	81.99	63.77	77.83	61.59	77.83
4	62.99	60.19	82.67	63.61	78.50	61.82	78.50
5	62.57	58.53	84.99	61.77	80.01	62.86	80.01
6	63.01	58.63	86.00	60.45	81.40	62.08	81.40
Average	62.4	58.8	82.9	62.8	78.1	61.6	78.10
RSD (%)	0.8	1.3	2.5	2.2	3.2	1.4	3.2

CONCLUSION: A gas chromatography with flame ionization detection method was described in this investigation to detect and monitor epichlorohydrin residual in linezolid drug. Method validation figures showed that the method developed is sensitive, precise, robust, and accurate in estimating epichlorohydrin. The process is simple, as it employs direct space extraction and most generally utilized flame ionization detection without derivatization steps for the sample. The proposed method can thus be used conveniently for regular quality control of epichlorohydrin in linezolid drug in the pharmaceutical laboratory.

ACKNOWLEDGEMENT: The authors thank the management of GVK Biosciences Private Ltd, Hyderabad, for facilities, co-operation, and support during the study.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Zurenko GE, Gibson JK, Shinabarger DL, Aristoff PA, Ford CW and Tarpley WG: Oxazolidinones: a new class of antibacterials. Current Opinion in Pharmacology 2001; 1(5): 470-76.
2. Seyed MRH, Tayebeh F and Mojdeh G: Linezolid: a review of its properties, function, and use in critical care. Drug, Design, Development and Ther 2018; 12: 1759-67.
3. Birmingham MC, Rayner CR, Meagher AK, Flavin SM, Batts DH and Schentag JJ: Linezolid for the treatment of multidrug-resistant, Gram-positive infections: experience from a compassionate-use program. Clinical Infectious Disease 2003; 36(2): 159-68.
4. Epichlorohydrin. PubChem, U.S. National Library of Medicine, National Center for Biotechnology Information. Accessed on May 2019. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/epichlorohydrin
5. Babu LC, Reddy RB, Gangaiah L, Madhusudhan G and Mukkanti K: A new and alternate synthesis of linezolid: An antibacterial agent. Der Pharma Chemica 2011; 3 (4): 219-26.
6. Epichlorohydrin, Immediately Dangerous to Life or Health Concentrations (IDLH), The National Institute for Occupational Safety and Health (NIOSH), Centers for disease control and prevention. Accessed on May 2019. Available at: https://www.cdc.gov/niosh/idlh/106898.html
7. European Medicines Agency, Guidelines on the Limits of Genotoxic Impurities. Accessed on May 2019. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-limits-genotoxic-impurities_en.pdf.
8. International Conference on Harmonization, S2(R1), Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, Washington, US, 2008.

9. International Conference on Harmonization, Q3A[R2], Impurities in new drug substances, Geneva, Switzerland, 2005.
10. International Conference on Harmonization, Q3C (R4), Impurities, Guideline for Residual Solvents, European Medicines Agency, London, UK, 2009.
11. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Genotoxic and Carcinogenic Impurities in Drug Substances and Products, Rockville, MD, 2008.
12. McGovern T and Jacobson-Kram D: Regulation of genotoxic and carcinogenic impurities in drug substances and products. Trends in Analytical Chem 2006; 25(8): 790-95.
13. Müller L, Mauthe RJ, Riley CM, Andino MM, Antonis DD, Beels C, DeGeorge J, De Knaep AG, Ellison D, Fagerland JA, Frank R, Fritschel B, Galloway S, Harpur E, Humfrey CD, Jacks AS, Jagota N, Mackinnon J, Mohan G, Ness DK, O'Donovan MR, Smith MD, Vudathala G and Yotti L: A rationale for determining, testing and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regulatory Toxicology and Pharmacology 2006; 44(3): 198-11.
14. Cohen E and Rizov N: Gas chromatographic determination of epichlorohydrin in workplace atmospheres. Chromatographia 1993; 37(1-2): 105-06.
15. Sarzanini C, Bruzzoniti MC and Mentasti E: Determination of epichlorohydrin by ion chromatography. Journal of Chromatography A 2000; 884(1-2): 251-59.
16. Lasa M, Garcia R and Millan E: A convenient method for epichlorohydrin determination in water using headspace – solid phase microextraction and gas chromatography. Journal of Chromatographic Science 2006; 44: 438-43.
17. Jerzy G and Grażyna W: Determination of epichlorohydrin in water and sewage samples. Talanta 2006; 70(5): 1044-50.
18. Kaliaperumal K, Govindasamy TA, Perumalsamy D and Karnam CP: Determination of residual epichlorohydrin in sevelamer hydrochloride by static headspace gas chromatography with flame ionization detection. Scientia Pharmaceutica 2010; 78(4): 835-46.
19. Shaik JV, Raveendra BG and Shakil SS: Estimation of epichlorohydrin content in pharmaceutical drug substances by capillary gas chromatography with flame ionization detection. Journal of Chemical and Pharmaceutical Research 2011; 3(6): 392-99.
20. International Conference on Harmonization, Q2R1, Validation of analytical Procedure: Text and Methodology, International conference on harmonization, Geneva, Switzerland 2005.

How to cite this article:
Devi DPVV, Narayanarao KMV, Shyamala P, Krishna RM and Prasad KS: Development and validation of gas chromatography method for the determination of genotoxic impurity, epichlorohydrin, in linezolid drug. Int J Pharm Sci & Res 2020; 11(3): 1284-89. doi: 10.13040/IJPSR.0975-8232.11(3).1284-89.

All © 2013 are reserved by the International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)