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Estimation of epichlorohydrin content in pharmaceutical drug substances by capillary gas chromatography with flame ionisation detection

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ABSTRACT

A rapid, economic, sensitive and reliable gas chromatographic method was developed and validated for the determination of residual Epichlorohydrin (ECH) in Pharmaceutical drug substances. This method utilized a Alltech AT-WAX GC column stationary phase consists of 100% polyethylene glycol, helium as carrier gas with flame ionization detection. The critical experimental parameters, such as, Column selection, injection volume and diluent were studied and optimized. The method was validated as per United States Pharmacopoeia (USP) and International Conference on Harmonization (ICH) guidelines in terms of detection limit (DL), quantitation limit (QL), linearity, precision, accuracy, ruggedness and robustness. A linear range from 0.6 to 3.4 µg/mL was obtained with the coefficient of determination (r2) 0.9985. The DL and QL of ECH were 0.15 µg/mL and 0.6 µg/mL, respectively. The recovery obtained for ECH was between 96.8% and 97.4%. This method was applied successfully to determine the content of Epichlorohydrin in several Pharmaceutical drug substances.

Keywords: Pharmaceutical drug substances, Carcinogen, Epichlorohydrin, Dimethyl sulphoxide, Validation, FID and GC.

INTRODUCTION

Epichlorohydrin is a versatile precursor in the synthesis of many organic compounds. Epichlorohydrin (ECH) is irritating and moderately toxic as well as carcinogenic, Toxic by inhalation, in contact with skin and if swallowed. ECH is chemically chloromethyloxirane. Epichlorohydrin (1-chloro-2,3-epoxypropane) is 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 [1]. Epichlorohydrin is mutagenic in most short-term assays and the

maximum contaminant level goal for Epichlorohydrin has been set at zero by the US Environmental Protection Agency (EPA). A review about the mutagenic and clastogenic effects of Epichlorohydrin is available [2]. Due to its toxicity, Epichlorohydrin has been listed among compounds dangerous to the water environment. No acceptable means of detecting Epichlorohydrin are currently available and the EPA requires water suppliers to use special treatment techniques to control its release into the environment [3-7].

The manufacturing process of bulk drugs consists of chemical synthesis extending to seven eight stages of processing involving different type of chemical reactions. In any one of the stage during synthesis of drugs if Epichlorohydrin is used or present by directly or indirectly determine the content of Epichlorohydrin using the above method.

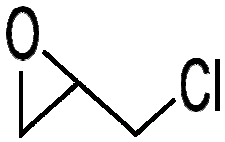

Fig 1. Epichlorohydrin Chemical Structure

EXPERIMENTAL SECTION

Chemicals and reagents

Spectroscopy grade dimethyl sulfoxide (DMSO), N,N-dimethyl formamide (DMF) and 1-methyl-2-pyrrolidone (NMP) were procured from Merck (Mumbai, India). Analytical grade N,N-dimethyl acetamide (DMAc) was from s.d Fine Chem. Ltd (Mumbai, India). (±) Epichlorohydrin (99+%) was purchased from Sigma-Aldrich. API drug substances are obtained from Pharma industries of Hyderabad, India.

Instrumentation

An Agilent 6890 GC (Agilent, Palo Alto, CA, USA) equipped with an auto sampler was used in the experiment; a straight glass injection liner with glass wool was obtained from Restek, (Restek and Bellefont, PA, USA). Data acquisition and processing were conducted using the waters Empower software.

Preparation of Stock, Standard and Test Solutions

The stock solutions of Epichlorohydrin were prepared by dissolving 30mg of Epichlorohydrin in 10mL solvent (stock-1). The 1000 µg mL^{-1} solution was prepared by transferring 1mL of the stock-1 solution into a 10mL volumetric flask and diluting to volume with sample solvent (stock-2), and further diluted to 75µL of the stock-2 solution into a 10mL volumetric flask and diluting to volume with sample solvent (7.5 ppm standard solution). 200µL of the stock-2 solution transferred into a 100mL volumetric flask and diluting to volume with sample solvent (LOQ solution) and 2.5mL of LOQ solution transferred into a 10mL volumetric flask and diluting to volume with diluent (LOD solution). The sample solution was prepared by accurately weighing about 1500 mg of the drug substance into a 5mL volumetric flask and dissolving in 5 mL of sample solvent. If sample solution becomes hazy filter the solution through 0.45µ nylon filter and collect clear solution in GC vials.

Chromatographic Conditions

The GC separation was conducted on an Alltech AT-WAX column with a dimension of 30 meter, 0.53 mm and a film thickness of 1.0 µm. Helium was used as carrier gas at a constant pressure of 4 psi. The GC oven temperature program utilized an initial temperature of 75 °C and

an initial holding time of 12 min, and then increased at 40°C per minute to 240 °C. The final temperature was held for 10 minutes. A flame ionization detection (FID) system was used. The detector temperature was set at 260 °C. The samples were injected with the Agilent 6890 series auto sampler. The inlet temperature was kept at 160 °C. The samples were injected in a split (1:0.5) mode with a 1.0 µL injection volume unless otherwise specified.

RESULTS AND DISCUSSION

Method Development and Optimization

The main challenge was to achieve the desired detection and quantitation limit using the most commonly available instrument, i.e. a gas chromatograph with a FID system. To obtain the desired sensitivity, one approach is to increase sample amount injected into the GC system. The adoption of a megabore capillary GC column (0.53 mm I.D.) with a high capacity bonded stationary phase seems to be the obvious choice. Suitable initial column temperature in combination with a moderate inlet temperature (160 °C) may allow a relatively large injection volume without significant deterioration in column efficiency.

The effect of injection volume on the quantitation of the Epichlorohydrin was investigated by injecting between 0.5 µL and 2 µL of the standard solution containing 2 ppm each of Epichlorohydrin [Fig.4-6]. The results show that the peak widths of Epichlorohydrin are independent of injection volume within the tested range. Further studies were done to select the suitable column which can retain the Epichlorohydrin at a suitable retention time (RT) and the suitable diluent which should not interfere at any unknown peak at Epichlorohydrin RT place. Based on certain trials AT-WAX column was chosen and Dimethyl sulphoxide diluent was most desirable which is not having any interference at Epichlorohydrin RT place [Fig.3]. Comparison of LOQ solution with blank was shown in Fig.7.

This method utilizes a dissolve-and-inject approach for the analysis. Several factors were considered in selection of a sample diluent, including the purity, its ability to dissolve the analyte, and its chemical compatibility with the compounds of interest. To detect the Epichlorohydrin at 0.50ppm level, the purity of sample solvent is critical. It has been observed in our laboratory that the Chromatography grade solvents are generally suitable.

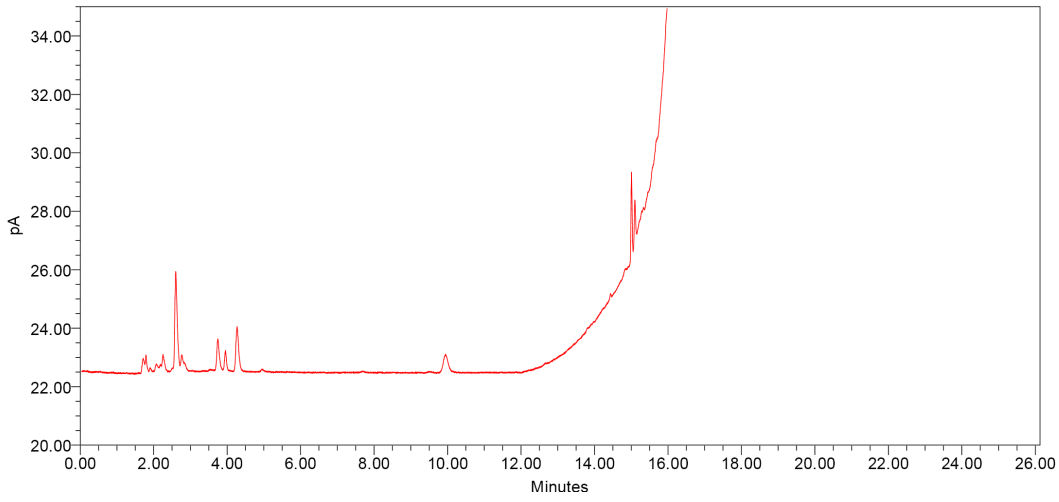

Fig.2: A typical chromatogram of Blank (dimethyl sulfoxide [DMSO])

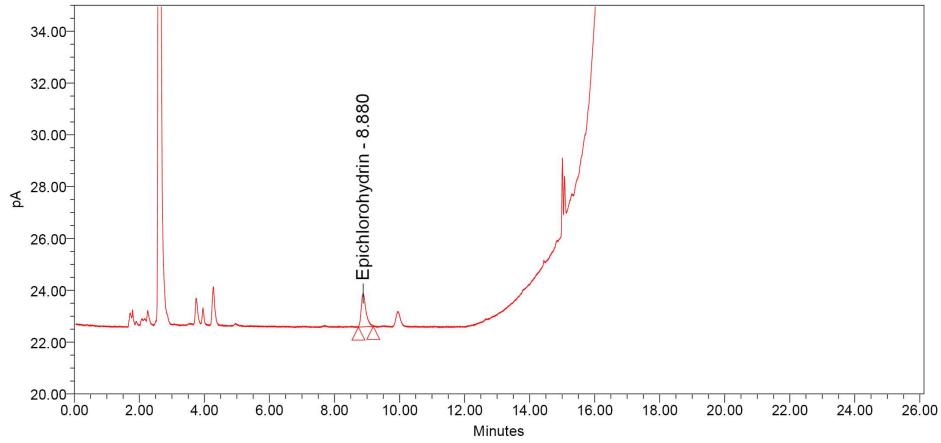

Fig.3: A typical chromatogram of Epichlorohydrin from 7.5 ppm standard solution

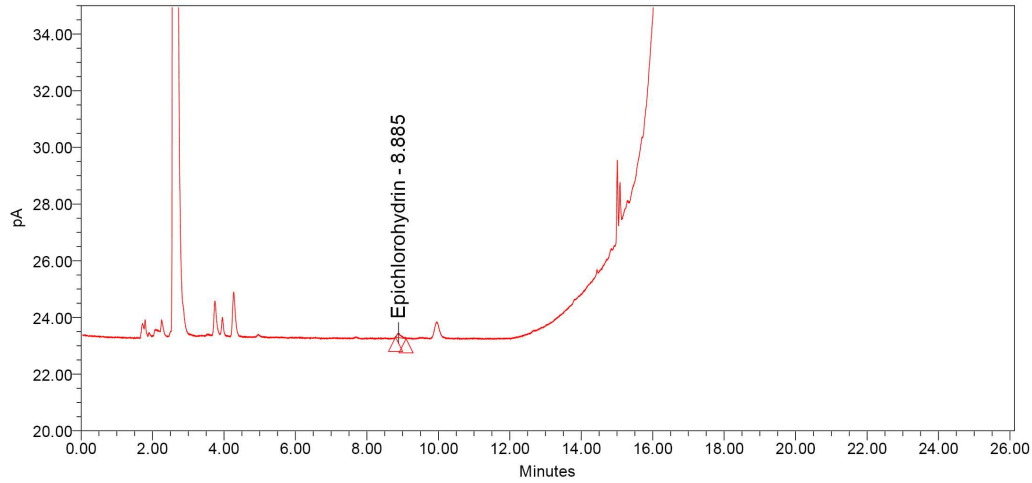

Fig.4: A typical chromatogram of Epichlorohydrin from LOD Solution

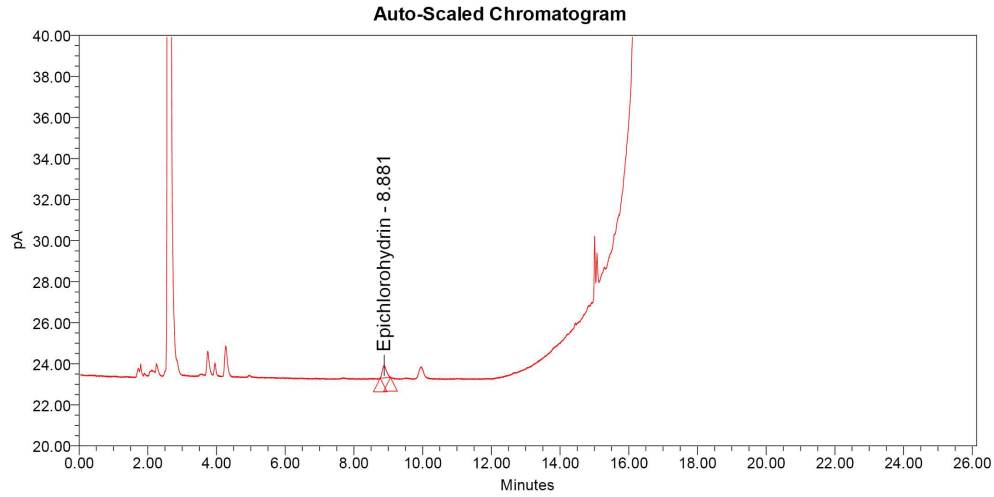

Fig.5: A typical chromatogram of Epichlorohydrin from LOQ Solution

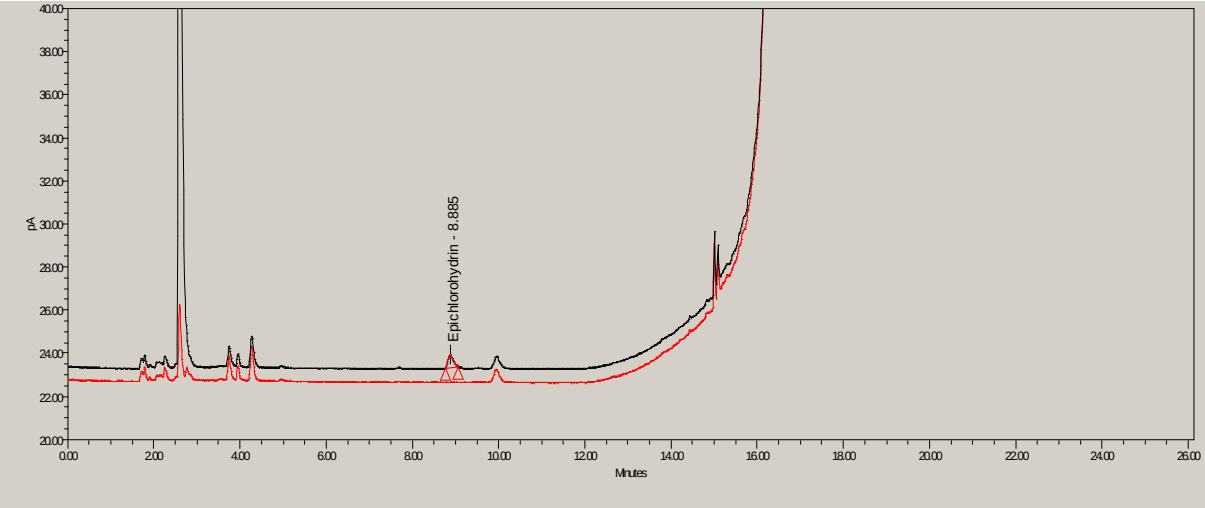

Fig.6: A typical chromatogram of Epichlorohydrin Comparison of LOQ Solution with blank

Method Validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines [8-9] the validated method parameters include accuracy, precision, linearity, ruggedness and robustness.

The detection limit (LOD) of the method for the Epichlorohydrin was estimated from a chromatogram of a solution containing about 0.50ppm. From the chromatogram, a signal-to-noise ratio of 2.7 was obtained. In the pharmaceutical industry, the quantitation limit (LOQ) was defined as the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The LOQ was determined to be 2.0ppm for Epichlorohydrin. From the chromatogram, a signal-to-noise ratio of 10.0 was obtained for LOQ solution. Precision and accuracy data for LOQ solution discussed below.

The experimental results also showed that this method has excellent precision without using an internal standard. Multiple injections (n=6) were made for the standard solutions containing 2.0ppm of Epichlorohydrin. For six injections of the solution, the RSD of the peak area of Epichlorohydrin was found to be 2.6% (Table 1). Accuracy of the method was determined by analyzing drug substance samples spiked with limit of quantification amount of the Epichlorohydrin. The recovery was 91.1% for Epichlorohydrin.
Linearity of the method was determined by preparing and analyzing a series of six (n=6) standard solutions to cover the concentration range of 2ppm – 11.25ppm (LOQ to 150%).The coefficient of determination (R^2) obtained for ECH was 0.9985 (Fig.8).

Accuracy of the method was determined by analyzing drug substance samples (n=3) spiked with 3.75ppm, 7.5ppm and 11.25ppm of the Epichlorohydrin. The recovery was 96.9%, 97.2% and 97.4% respectively for Epichlorohydrin (Table 2).

Ruggedness of the method was performed by doing precision study for the standard solution with different column, different system and different analyst and the percentage RSD for the Epichlorohydrin peak area is about 4.7.

Robustness of the method was checked by varying the column oven temperature from 75°C to 70°C and 80°C and the column flow from 4 psi to 3.8 psi and 4.2 psi. Precision study was done

in the above modified conditions for the standard solution and the percentage RSD for the Epichlorohydrin peak area was tabulated below (Table 3).

Solution study was performed for 24 hours and found the solution to be stable. The selectivity of the method also demonstrated by injecting the process related solvents which are used for preparing API drug and those are well separated with Epichlorohydrin. This method can be used for all API drugs. If any of the API drug process related solvent interfering at the RT of Epichlorohydrin RT then slightly modify the flow of the carrier gas so that it can separate with the Epichlorohydrin peak.

Table 1: Precision at LOQ

Preparation No	Retention time	Epichlorohydrine (Area)
01	8.811	5.10
02	8.802	5.01
03	8.805	5.19
04	8.808	4.98
05	8.805	4.91
06	8.812	4.82
Average	8.807	5.00
Standard deviation	0.004	0.13
%RSD	0.04	2.64

Table 2: Accuracy Results

Level of Accuracy	S.No.	ECH added (µg/mL)	ECH recovered (µg/mL)	% ECH Mean ± SEM (n=3)
at 50%	1	0.302	0.293	96.86 ± 0.26
	2	0.313	0.301	
	3	0.307	0.299	
at 100%	1	0.750	0.731	97.21 ± 0.10
	2	0.756	0.735	
	3	0.755	0.732	
at 150%	1	1.520	1.480	97.36 ± 0.02
	2	1.510	1.470	
	3	1.530	1.490	

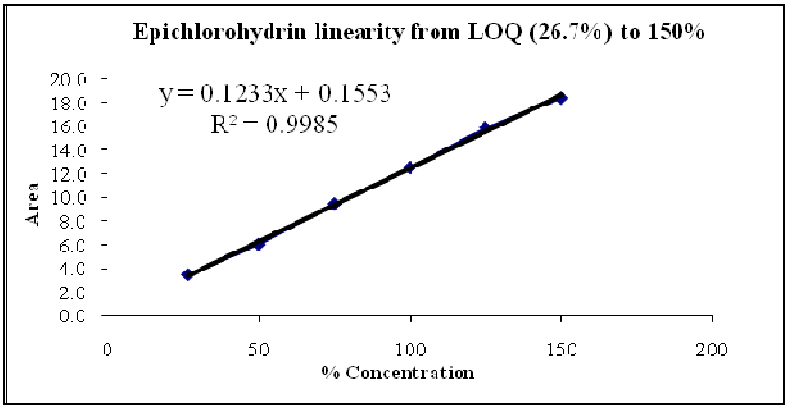

Fig.7: Linearity curve

Table 3: Robustness Data

Precision at different conditions

Preparation No	Oven temperature (70°C)		Oven temperature (80°C)		Carrier gas flow (3.8 psi)		Carrier gas flow (4.2 psi)	
	Epichlorohydrin		Epichlorohydrin		Epichlorohydrin		Epichlorohydrin	
	Area	RT	Area	RT	Area	RT	Area	RT
01	13.22	10.349	13.67	7.657	13.30	9.827	13.71	8.022
02	13.20	10.347	14.11	7.673	13.00	9.816	14.11	8.027
03	13.52	10.350	13.85	7.658	12.08	9.830	13.93	8.029
04	13.44	10.347	14.02	7.662	13.39	9.828	13.65	8.029
05	13.19	10.323	13.76	7.671	12.89	9.835	13.79	8.029
06	13.53	10.336	14.19	7.660	13.24	9.824	14.00	8.034
Average	13.35	10.342	13.93	7.664	12.98	9.827	13.87	8.028
Standard deviation	0.16	0.01	0.21	0.01	0.48	0.01	0.18	0.004
%RSD	1.23	0.102	1.47	0.089	3.70	0.065	1.28	0.048

CONCLUSION

A simple and sensitive GC method has been developed and validated for the trace level analysis of Epichlorohydrin in Milnacipran hydrochloride. Compared with the previously reported methodologies, this method utilizes a FID detector, which is readily available in most of the quality control testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 0.50ppm of Epichlorohydrin and can quantify up to 2.0ppm. It is having less run time and avoided headspace and splitlless techniques which create lot of interference and baseline noise.

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