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Trace level analysis of epichlorohydrin in pharmaceuticals by capillary gas chromatography with flame ionization detection

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ABSTRACT

A capillary gas chromatographic method using flame ionization detection was developed and validated for the trace analysis (ppm level) of Epichlorohydrin in pharmaceutical drug substance and drug product. The method utilizes a capillary column with bonded and 6% Cyanopropylphenyl-94% Dimethyl Polysiloxane (As per USP G43) stationary phase. A dissolve-and-injection approach was adopted for sample introduction in a splitless mode. Dichloromethane is used as sample solvent. A limit of detection of about 0.18 ppm and limit of quantitation of about 0.51 ppm were achieved for the Epichlorohydrin in drug substance and drug product samples. The method optimization and validation are also discussed in this paper.

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KEYWORDS

Pharmaceutical analysis of
Epichlorohydrin;
Dichloromethane;
Validation;
FID;
GC.

INTRODUCTION

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]. Their presence in the pharmaceutical products may be the result of leftover starting materials, or formed as by-product. Official guidelines have been established, the concentration of the compound is expected to be controlled at a level less than or equal to 1ppm. 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].

Literature methods for the determination of Epichlorohydrin in water samples are based on Ion chromatography and ICMS but IC or ICMS preceded by time-consuming. Recently, an analytical method based on solid-phase extraction and subsequent GC determination has been presented^[8-13]. To our knowledge, no dissolve-and-injection approach Gas chromatographic method is available for the determination of Epichlorohydrin in pharmaceutical products, it is simple and accurate technique.

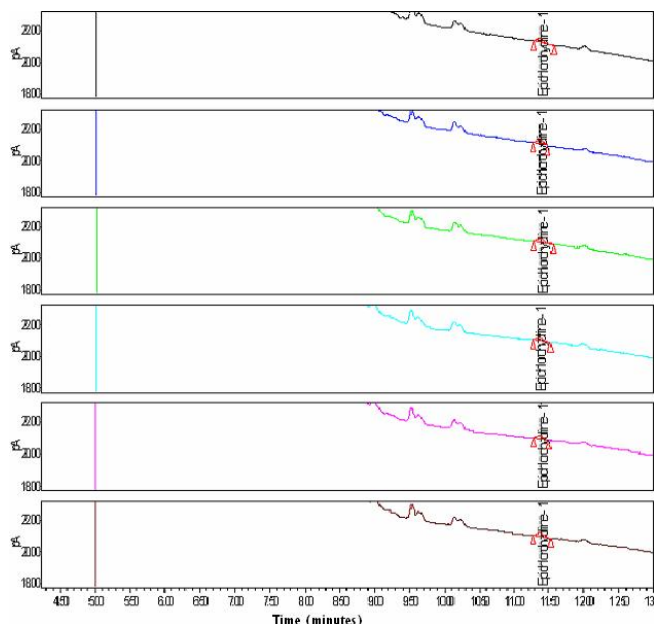
Note

The pure Epichlorohydrin is liquid at ambient temperature with a boiling point around 117.9 °C. Therefore, it is feasible to analyze and quantify this compound by gas chromatography. The analysis of the Epichlorohydrin using HPLC is not straightforward because of the specific physical and chemical properties of this compound.

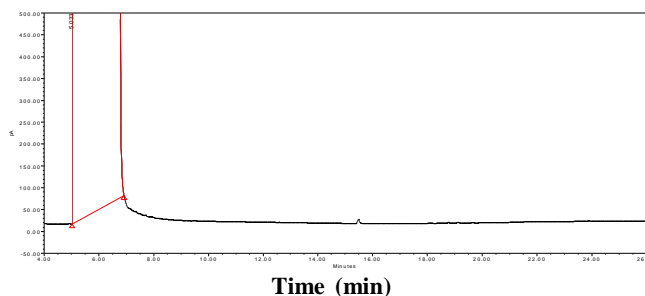
The aim of this work was to develop a reliable technique for Epichlorohydrin determination based on Gas chromatography. The method has been optimized

through the parameters involved in the dissolve-and-injection. The method developed, coupled with a preconcentration procedure, allowed one to obtain, after procedure optimization, a detection limit of 0.18ppm and quantification limit is 0.51 ppm (Figure 1) Epichlorohydrin.

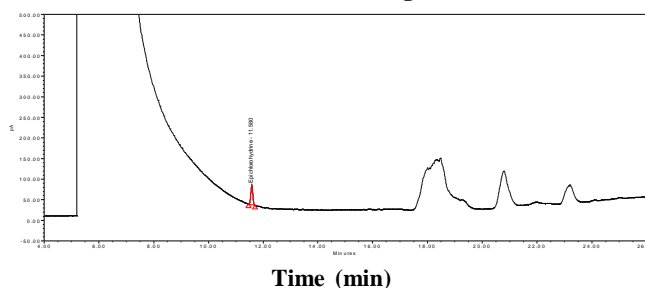
This short communication describes a simple and sensitive method for the determination of Epichlorohydrin in pharmaceuticals using capillary GC with flame ionization detection (FID).



(a) Epichlorohydrin LOQ precision:



(b) Blank chromatogram.



(c) Epichlorohydrin standard (0.51ppm) chromatogram:

Figure 1

EXPERIMENTAL

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, Bellefont, PA, USA). Data acquisition and processing were conducted using the waters Empower software.

Chemicals

Epichlorohydrin was purchased from Aldrich Chemical (Milwaukee, WI, USA). Chromatography grade equivalent Dichloromethane was obtained from Merck. This study also involves to proprietary Dr. Reddy's Laboratories Pharmaceutical Research & Development compounds.

Preparation solutions

The stock solutions of Epichlorohydrin were prepared by dissolving 100mg of Epichlorohydrin in 10mL solvent (stock-1). The diluted stock-1 solution was prepared by pipetting 102 μ L of the stock-1 solution into a 10mL volumetric flask and diluting to volume with sample solvent (stock-2), and further diluted to 15 μ L of the stock-2 solution into a 10mL volumetric flask and diluting to volume with sample solvent (LOQ solution). 2.83mL of LOQ solution into a 10mL of diluent (LOD solution). The sample solution was prepared by accurately weighing about 300 mg of the drug substance into a 2mL GC vial and adding 1.0 mL of sample solvent.

Chromatographic conditions and equipment

The GC separation was conducted on an Alltech

DB-624 (G 43) column with a dimension of 30 meter, 0.32 mm and a film thickness of 1.8 μm . Helium was used as carrier gas at a constant pressure of 6 psi. The GC oven temperature program utilized an initial temperature of 40 $^{\circ}\text{C}$ and an initial holding time of 2 min, and then increased at 10 $^{\circ}\text{C}$ per minute to 240 $^{\circ}\text{C}$. The final temperature was held for 4 minutes. A flame ionization detection (FID) system was used. The detector temperature was set at 280 $^{\circ}\text{C}$. The samples were injected with the Agilent 6890 series auto sampler. The inlet temperature was kept at 130 $^{\circ}\text{C}$. The samples were injected in a splitless mode with a 1 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 (130 $^{\circ}\text{C}$) 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 3 μL of the standard solution containing 1ppm each of Epichlorohydrin. The results show that the peak widths of Epichlorohydrin are independent of injection volume within the tested range. Further studies were not done to determine the maximum injection volume that the chromatographic system could handle because interfering peaks from the sample solvent started being detected when the injection volume was greater than 2 μL in our experiments. An injection volume of 1 μL was chosen for this method as it will not over load the column.

The effect of inlet temperature on baseline drift and thermal stability of Epichlorohydrin drug substance was thoroughly investigated. The inlet temperature was varied from 100 to 200 $^{\circ}\text{C}$. An aliquot of 1 μL of the sample

was injected in the splitless mode. The results shows that if the inlet temperature more than 160 $^{\circ}\text{C}$, it is observed that drift in the baseline and slight degradation of Epichlorohydrin, which will effect the quantification of Epichlorohydrin. An inlet temperature of 130 $^{\circ}\text{C}$ was chosen, which allowed smooth baseline.

This method utilizes a dissolve-and-inject approach for the analysis. Several factors were considered in selection of a sample solvent, including the purity, its ability to dissolve the analyte, and its chemical compatibility with the compounds of interest. To detect the Epichlorohydrin at 0.18ppm level, the purity of sample solvent is critical. It has been observed in our laboratory that the Chromatography grade solvents are generally suitable. The tested sample concentration of drug substances was in the range of 100–300 mgmL^{-1} . The Epichlorohydrin showed reasonable stability in the organic solutions, so dichloromethane was used as sample diluent and more over all drug substances soluble in dichloromethane. This is important because many pharmaceuticals are in without salt forms, which shows high solubility in pure organic solvents.

Method validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines^[14,15] the validated method parameters include specificity, accuracy, precision, and solution stability.

Sensitivity

The detection limit (LOD) of the method for the Epichlorohydrin was estimated from a chromatogram of a solution containing about 0.18ppm. From the chromatogram, a signal-to-noise ratio of 2.0 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 0.51 ppm for Epichlorohydrin based on the precision and accuracy data discussed below.

The experimental results also show that this method has excellent precision without using an internal standard. Multiple injections ($n=6$) were made for the standard solutions containing 0.51 ppm of Epichlorohydrin. For six injections of the solution, the RSD of the peak area of Epichlorohydrin was 4.9%.

Note

Accuracy

Accuracy of the method was determined by analyzing drug substance samples spiked with limit of quantification amount of the Epichlorohydrin. The recovery was 106% for Epichlorohydrin. Because this method uses the dissolve-and-inject approach, for every sample injection, about 300 mg of the drug substance is introduced in the injection port. The accumulation of drug substance may have negative effect on the recovery. Therefore the injection liner should be replaced after every sequence of 20–25 injections.

Linearity

Linearity of the method was determined by preparing and analyzing a series of six ($n=6$) standard solutions to cover the concentration range of 0.51 ppm – 6.0 ppm. Regression analysis of the peak area versus concentration data yields an $R^2 > 0.996$.

Accuracy/Recovery

Accuracy of the method was determined by analyzing drug substance samples ($n=4$) spiked with 2 ppm, 3 ppm, 4 ppm and 6 ppm of the Epichlorohydrin. The recovery was 101%, 98.4%, 105.1%, and 95.3% respectively for Epichlorohydrin.

Ruggedness

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 4.0.

Robustness

Robustness of the method was checked by varying the column oven temperature from 40°C to 35°C and 45°C and the column flow from 6 psi to 6.5 psi and 5.5 psi, different day and analyst. Precision study was done in the above modified conditions for the standard solution and the percentage RSD for the Epichlorohydrin peak area is about 6.0.

Solution stability

Solution study was performed for 24 hours and found the solution to be stable.

CONCLUSION

A simple and sensitive GC method has been developed and validated for the trace level analysis of Epichlorohydrin in pharmaceuticals. 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 0.18 ppm of Epichlorohydrin and can quantify up to 0.51 ppm in pharmaceutical products.

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