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Validated Chiral High Performance Liquid Chromatographic Method for Enantiomeric Separation of Epichlorohydrin on Immobilized Amylose Based Stationary Phase

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ABSTRACT

Chiral Liquid Chromatographic (CLC) method has been established and authenticated for the enantiomeric resolution of epichlorohydrin. Chiral resolution was achieved on Chiralpak-IA immobilized amylose based stationary phase using an eluent consisting of n-hexane:2-propanol (100:2 v/v) and flowing through column at rate of 1.0 ml/min. The column effluent was examined by using UV detector at 205 nm. Resolution between S-(+)-Epichlorohydrin (SE) and R-(-)-Epichlorohydrin (RE) was found to be ≥ 5. The method was authenticated for the quantification of RE in SE. The detector response for RE was proportionate over the concentrations range of 3-40 µg/ml. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for RE were 0.95 µg/ml and 3.2 µg/ml respectively. Average recovery of the RE was in the range of 96.20% to 101.99%. Analytical solutions of both enantiomers in diluent were found to be stable for a day. The proposed method was found to be precise, accurate and robust for quantitative analysis of RE in SE.

Keywords: Epichlorohydrin, Chiral LC, Immobilized amylose based stationary phase, Normal phase, Method validation

INTRODUCTION

S-(+)-Epichlorohydrin (1-chloro-2,3-epoxypropane), an enantiomer is used as chiral building block in the synthesis of biologically active isomers of oxazolidinones and other bioactive compounds [1-7]. The structure of epichlorohydrin is displayed in Figure 1. The quantification of RE as unwanted impurity in SE is very important aspect for obtaining enantiomerically pure new drug substances. The literature reveals that very few GC or enzymatic [8-12] methods are reported for chiral resolution of epichlorohydrin. The reported GC method uses chiral GC column (G-DM) for enantiomeric separation of epichlorohydrin with resulting peak resolution of 1.35. Thus the literature suggests a need of simple and sensitive method for enantiomeric separation of epichlorohydrin and for quantification of traces of chiral impurity.

CLC offers many advantages including a wide spectrum of commercially available CSPs to choose from multiple separation modes, excellent preparative potential, high sensitivity, accuracy, robustness and transferability. So far to our knowledge there is no validated Chiral Liquid Chromatographic (CLC) method reported for quantification of RE in SE and vice a versa. Polysaccharide based stationary phases are fairly prevalent with extensive capability for direct resolution of enantiomers [13-15]. Attempts were made to develop CLC method for enantiomeric resolution of epichlorohydrin using immobilized amylose based stationary phase. This article mainly deals with quantification of RE in SE using normal phase liquid chromatographic method. Developed method was authenticated for specificity, linearity, precision, recovery, Limit of Detection (LOD) and Limit of Quantification (LOQ) parameters.

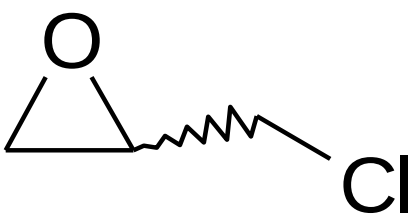

Figure 1: Structure of epichlorohydrin

MATERIALS AND METHODS

R-(-)-Epichlorohydrin (99.0%) and S-(+)-Epichlorohydrin (99.0%) were procured from Fluka (USA). High Performance Liquid Chromatography (HPLC) grade n-hexane and 2-propanol were bought from Rankem fine chemicals (Mumbai, India). The HPLC system (Shimadzu, Japan) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and UV detector was employed. The detector output was processed using 10A-VP series with LC-10 software. The diluent for solution preparation was prepared by mixing n-hexane and 2-propanol in ratio of 100:2 v/v.

Standard solution preparation

Solution of RE was prepared having concentration of 2 mg/ml in mobile phase. About 20 mg of SE was weighed separately in 10 ml of volumetric flask, added 5.0 ml of mobile phase to dissolve, added 0.1 ml of RE stock solution and diluted to volume with diluent. A solution containing 0.002 mg/ml of RE and 2.0 mg/ml of SE was prepared in diluent.

Test solution preparation

A solution containing 2.0 mg/ml of SE was prepared in diluent.

Chromatographic condition

The LC column used was 250 × 4.6 mm ID Chiralpak-IA (Daicel Chemical Industries, Ltd., Tokyo, Japan) packed with 5 µm particles. The eluent used was n-hexane: 2-propanol (100:2 v/v). The flow rate of the eluent was 1.0 ml/min. The column was maintained at 30°C and the effluent was monitored at a wavelength 205 nm. Injection volume was 20 µl.

Method development

The goal of this study was to resolve the enantiomers chromatographically and accurately quantify RE in SE. A standard solution containing both enantiomers as described above was used for the method development. Considering suitable physico-chemical properties of analyte, initially the efforts were made to develop a method on gas chromatography using β- and γ-Cyclodextrin based chiral stationary phases (30 mm × 0.25 mm × 0.25 µ, Supelco analytical). Epichlorohydrin enantiomers did not resolve on GC by using above mentioned chiral columns. Other option of liquid chromatographic separation using chiral stationary phases (CSP) was employed. Different CSP's like Chiralcel-OJH, Chiralcel-ODH and Chiralcel-IB (cellulose based CSP), Chiralpak-ADH and Chiralpak-IA (Amylose based CSP) together with different combination of mobile phases were employed. No separation of Epichlorohydrin enantiomers was achieved on Chiralcel OJ-H, Chiralcel OD-H and Chiralpak-IB CSP's. A little resolution was obtained on Chiralpak-ADH column. Chiralpak IA was found to be the best among all. Mobile phase composition was optimized that would give the acceptable resolution and discrimination for two enantiomers. Excellent separation was achieved on Chiralpak-IA column using mobile phase of n-hexane:2-propanol (100:2 v/v). The above newly optimized method was further authenticated for dependability.

Method validation

Specificity

Specificity was evaluated by injecting diluent as blank solution, individual isomers and spiked solution of the RE in SE.

Precision

The precision of the method was evaluated by analyzing six replicates of SE (at concentration 2 mg/ml) solution.

Linearity

The solutions of RE were analysed over the concentrations range of 3-40 µg/ml. Linearity graph was obtained by plotting the peak area against concentration.

Accuracy/recovery

Accuracy of the method was warranted by measuring recovery of the spiked amount of RE in pre-analyzed sample of SE at three levels in the range of 50 to 150%.

Limit of detection (LOD) and *limit of quantification* (LOQ)

LOD and LOQ of RE were determined by signal to noise ratio method.

Robustness

Robustness was gauged by determining LC resolution between two enantiomers. Flow rate was altered by ± 10%, column temperature by ± 5°C and proportion of 2-propanol in eluent by ± 2%.

Solution stability and mobile phase stability

Stability of RE and SE in the analytical solution was studied by allowing it in tightly capped volumetric flask at room temperature for a day. Content of RE in SE was checked at 6 h intervals upto 24 h.

RESULTS AND DISCUSSION

Enantiomeric resolution of epichlorohydrin was successfully obtained on Chiralpak-IA, which is a Tris-3,5-tris-dimethylphenylcarbamate derivative of immobilized amylose coated on silica. A representative chromatogram of SE spiked with RE is shown in Figure 2 depicting an excellent LC resolution (Rs=6.9) between two enantiomers, symmetric peak shape with peak tailing factor of 1.8.

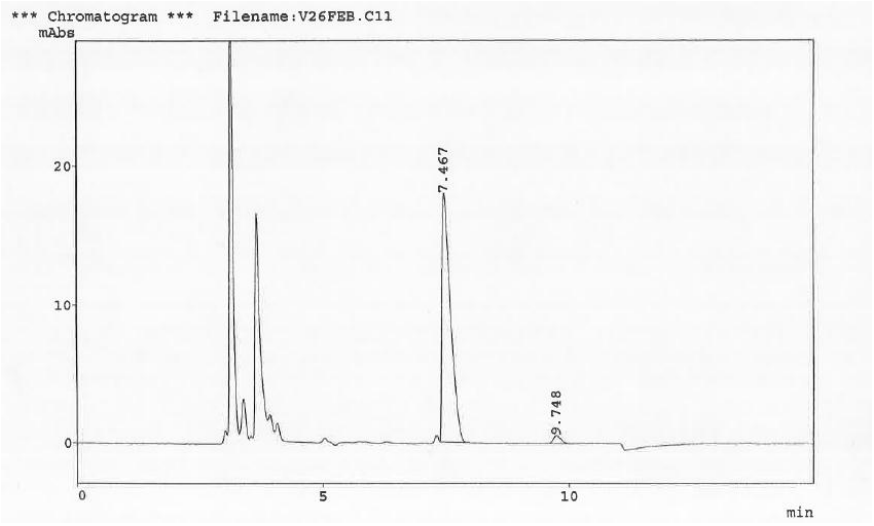

Figure 2: Representative chromatographic resolution of S-(+)-Epichlorohydrin (Retention time: 7.47 min) spiked with R-(-)-Epichlorohydrin (Retention time: 9.75 min)

Enantiomeric separation may be due to the interaction between the carbamate group on the CSP and epoxide group of analyte through hydrogen bonding. System suitability parameters and results obtained are summarized in Table 1.

Table 1: System suitability data

Analyte	R_t	α	Rs	N	T
S-(+)-Epichlorohydrin	7.48	-	-	10457	1.9
R-(-)-Epichlorohydrin	9.75	1.4	6.93	15338	0.99

R_t-Retention time; α-Enantioselectivity; Rs-USP resolution; N-Number of theoretical plates (USP tangent method) and T-USP tailing factor

It was observed that from diluent there were no interfering peaks eluting at the retention time of SE and RE. The representative chromatogram of test batch of SE is displayed in Figure 3.

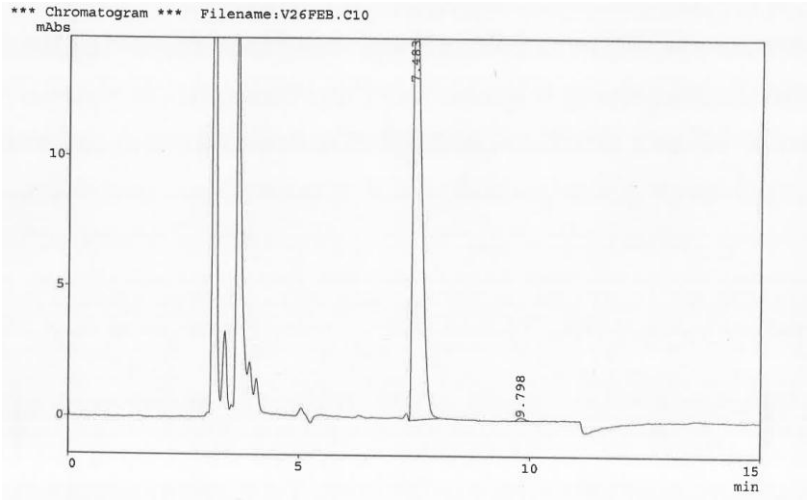

Figure 3: Representative chromatogram of commercial lot of S-(+)-Epichlorohydrin

The method was found to be precise with Relative Standard Deviation (RSD) less than 1.0% for RE content. The detector response was found to be proportionate in the range of 3-40 µg/ml of RE with correlation of 0.999 and regression equation Y=11.558x–52.1. Recovery was found to be in the range of 96.20% to 101.99% for RE, results are shown in Table 2.

Table 2: Recovery results of R-(-)-Epichlorohydrin

Level	Amount spiked (%)	Amount found (%)	Recovery (%)
0.0	0.0	0.45	-
50	0.52	0.95	96.20
100	1.12	1.55	98.21
150	1.51	1.99	101.99
Average recovery (%)			**98.8**

LOD and LOQ for RE was determined by signal to noise ratio method. The LOD and LOQ values are shown in Table 3.

Table 3: Validation results of the method

Validation parameter	Results
Repeatability (n=6) %RSD	
Peak area of S-(+)-Epichlorohydrin	0.8
Peak area of R-(-)-Epichlorohydrin	0.7
LOD and LOQ of R-(-)-Epichlorohydrin	
LOD (µg/ml)	0.95
LOQ (µg/ml)	3.2
Linearity of R-(-)-Epichlorohydrin peak	
Calibration range (µg/ml)	3.0–40
Correlation coefficient	0.9998

Epichlorohydrin enantiomers were found to be stable in diluent for a day. The optimized method was found to be robust as no significant impact was observed on the peak resolution of the two enantiomers due to limited variations in chromatographic conditions. The robustness data is revealed in Table 4.

Table 4: Robustness of the chiral LC method

Parameter	Rs	%RSD
Flow rate (ml/min)		
0.9	7.12	0.66
1.0	6.93	0.58
1.1	6.11	0.75
Column temperature (°C)		
25	7.20	0.79
30	6.93	0.80
35	5.99	0.69
Mobile phase (% of 2-propanol)		
1.5	7.60	0.90
2	6.93	0.80
2.5	5.57	0.56

Applicability of method

The newly optimized method was employed for quantitation of RE in SE. The SE is being used as key raw material for synthesis of biologically active new chemical entities. The newly optimized method was successfully used for analysis of commercial samples of SE. Five different batches of SE were analyzed wherein the RE presence was found in the range of 0.30-0.55% w/w.

CONCLUSION

A simple, specific, precise and accurate normal phase chiral LC method has been optimized and authenticated for the resolution of Epichlorohydrin enantiomers. Chiralpak-IA (Immobilized amylose based stationary phase) column was found to be enantioselective for the analysis of Epichlorohydrin enantiomers. The method was completely validated displaying satisfactory data for all the method validation parameters. The newly developed method was successfully used for accurate measurement of R-(-)-Epichlorohydrin (RE) content in commercial supply of S-(+)-Epichlorohydrin (SE).

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