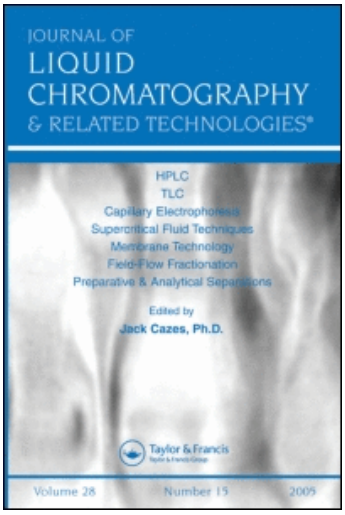

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Enantiomeric Separation of Several Oxirane Derivatives by High Performance Liquid Chromatography on Polysaccharide-Based Chiral Stationary Phases

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[2]Department of Chemistry, Shandong University, Jinan, P. R. China
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[4]Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Cairo, Egypt

Abstract: Five polysaccharide based chiral stationary phases have been used for separation of enantiomers of fourteen heterocyclic oxiranes. The polysaccharide based chiral stationary phases used in this study are Chiralak AD, Chiralpak AS, Chiralcel OD, Chiralcel OG, and Chiralcel OJ. From the chiral separation results, the chiral separation ability for the selected compounds show the order of Chiralpak AD > Chiralcel OD > Chiralcel OJ > Chiralcel OG > Chiralpak AS. Chiralcel OD appears to be quite versatile, since 8 out of 11 oxiranes with π-aromatic system were successfully resolved, yet no resolution was obtained for those oxirane derivatives, which lacks the π-aromatic system. Although Chiralcel OD is also versatile, it was not as effective; since the separation factors (α) are much smaller. The results indicate that dipole interactions have a strong impact on the retention mechanism, and extended π systems are essential. The spatial arrangement of the substituent groups around the analyte stereogenic center plays an important role in enantiomeric separations. The closer a group is to the chiral center, the more likely is the chiral recognition and enantioselectivity.

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Keywords: Chiral recognition mechanisms, Chiral separation, Oxiranes, Polysaccharide based CSPs

INTRODUCTION

Enantiomeric separation methods using high performance liquid chromatography (HPLC) have been extensively developed in the separation and analysis of racemic drugs. This technique is currently used to achieve chiral separation of racemic mixtures by HPLC using chiral stationary phases (CSPs) because it is rapid and efficient for the resolution of racemic mixtures. Several CSPs are now commercially available to allow the direct separation and analysis of drug enantiomers and racemates.[1] Polysaccharide type CSPs are among the commonly employed phases used for the separation and enantiomeric purity determination.[2,3]

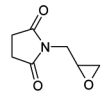
The alkylating capacity of epoxy functions is well known[4–6] and the antineoplastic activity of bifunctional epoxides has been reported.[7–9] The investigation of active antitumor agents containing higher numbers of epoxy groups in their structure has been performed.[10] On the other hand, it is known that the chemical reactivity of epoxides is strongly dependent on the substituents and their location of the oxirane ring in the molecule.[11] A number of heterocycles containing a pyridine type nitrogen atom and an oxirane ring have been designed as antitumor compounds.

In this paper, we describe the separation of a series of racemic oxiranes enantiomers on five polysaccharide based chiral stationary phases. The influence of property and enantioselectivity of substituents on chiral separation have been investigated. The chiral recognition mechanisms involved between those analytes and the chiral selector used in this study are also discussed.

EXPERIMENTAL

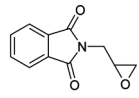
Materials

A series of fourteen oxirane derivatives were synthesized by the Institut of Organic Chemistry, Ebarhard-Karls-University Tübingen, Germany.[12] The structure of the compounds is presented in Figure 1. These compounds were dissolved in isopropanol and then diluted with the mobile phase. Solutions with approximate concentration of 0.1 mg mL^{-1} in eluent solvent were used for injection. All solvents were filtered by a 0.5 μm filter and degassed in helium.



1

1-(2,3-epoxypropyl)-2,5-pyrrolidinedione



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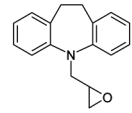

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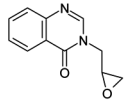
5-oxiranylmethyl-10,11-dihydro-5H-dibenz[b,j]azepine



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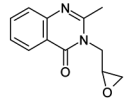

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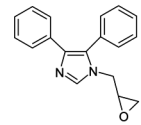
2-methyl-3-oxiranylmethyl-3H-quinazolin-4-one



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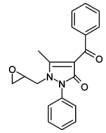
1- oxiranylmethyl-4,5-
2- diphenyl-1H-imidazole



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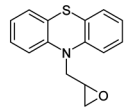
4-benzoyl-5-methyl-1-oxiranylmethyl-2-phenyl-1,2-dihydro-pyrazol-3-one



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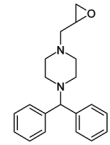
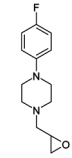
10-oxiranylmethyl-10H-phenothiazine



9

1-benzhydryl-4-oxiranylmethyl-piperazine



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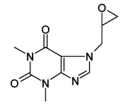
1-(4-fluoro-phenyl)-4-oxiranylmethyl-piperazine

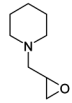


11

1,3-dimethyl-7-oxiranylmethyl-3,7-dihydro-purine-2,6-dione

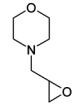
12

1-oxiranylmethyl-pyrrolidine



13

1-oxiranylmethyl-piperidine



14

4-oxiranylmethyl-morpholine

Figure 1. Names and structures of 14 oxirane derivatives used in this study.

Apparatus

The chromatography was performed with Spectra Physics SP8800 HPLC Pump, Spectra Physics SP8880 Autosampler, Spectra Physics Spectra 100 Detector, and Spectra Physics Chromjet Integrator (Irvine, CA, USA).

Chromatography Conditions

The amylose tris[(S)-α-methylbenzyl carbamate] chiral stationary phase Chiralpak AS (250 × 1 mm ID), amylose tris(3,5-dimethylphenyl carbamate) chiral stationary phase Chiralpak AD (250 × 2 mm ID), cellulose tris(3,5-dimethylphenyl carbamate) chiral stationary phase Chiralcel OD (250 × 2 mm ID), cellulose tris(4-chlorophenyl carbamate) chiral stationary phase Chiralcel OG (250 × 2 mm ID), and cellulose tris(4-methylbenzoate) chiral stationary phase Chiralcel OJ (250 × 2 mm ID) were from Grom (Herrenberg-Kayh, Germany). The mobile phase compositions were 10% or 30% of isopropanol in *n*-heptane with a different column. The flow rate was maintained at 0.5 mL min^{-1}.

RESULTS AND DISCUSSION

Enantiomeric Separation of 14 Oxirane Derivatives on Five Polysaccharide Based Chiral Stationary Phases by HPLC

Based on the impact of different substructures on the separation data, the 14 oxirane derivatives (Figure 1) are classified in two classes, according to their chemical structure, one class where the substructure possesses a π aromatic ring system, and the other class which does not possess a $\tilde{\pi}$ aromatic ring system. The separation data are compiled in Tables 1–5 for the 14 analytes where the π aromatic ring system is present and absent, respectively.

The quality of an HPLC separation is adequately described in terms of the resolution achieved. In order to evaluate the chromatographic performance of the five selected columns in the enantiomeric separation of the 14 oxiranes, several factors were investigated. The retention factors (k'), the separation factor of the enantiomers, α, and the resolution factor (R_s). Various mathematical functions have been proposed to evaluate the separation quantitatively.[13–14] Thus, the retention factor k' is given as in Equation (1)

$$k' = (t_R - t_0)/t_0 \quad (1)$$

Table 1. Retention times (t); retention factors (k'), separation factors (α) and resolution factors (R_S) for HPLC of 14 oxiranes on a CHIRAL AD stationary phase; mobile phase: heptane/iso-propanol (70/30), flow rate: 0.5 mL/min, $t_0 = 1.329$

Compounds	t_1	t_2	k'	α	R_S
1	4.446	7.574	3.522	2.004	3.476
2	3.125	3.811	1.609	1.382	1.247
3	1.889	–	0.421	1.000	0
4	3.395	4.873	2.111	1.715	2.111
5	2.929	5.362	2.119	2.521	3.476
6	2.753	–	1.710	1.000	0
7	2.859	5.209	2.035	2.536	3.357
8	2.118	2.390	0.696	1.345	1.360
9	1.878	2.075	0.478	1.359	0.985
10	1.820	1.999	0.437	1.365	0.895
11	1.978	–	0.488	1.000	0
12	4.441	–	2.342	1.000	0
13	1.495	–	0.125	1.000	0
14	1.645	–	0.238	1.000	0

Table 2. Retention times (t); retention factors (k'), separation factors (α) and resolution factors (R_S) for HPLC of 14 oxiranes on a CHIRAL AS stationary phase; mobile phase: heptane/iso-propanol (90/10), flow rate: 0.5 mL/min, $t_0 = 0.479$

Compounds	t_1	t_2	k'	α	R_S
1	5.217	–	9.891	1.000	0
2	1.584	–	2.307	1.000	0
3	0.789	–	0.647	1.000	0
4	2.700	–	4.637	1.000	0
5	1.491	–	2.113	1.000	0
6	1.919	–	3.006	1.000	0
7	6.782	–	13.159	1.000	0
8	0.916	–	0.912	1.000	0
9	0.673	–	0.405	1.000	0
10	1.275	2.184	2.611	2.142	0.909
11	3.235	–	5.754	1.000	0
12	0.642	–	0.340	1.000	0
13	0.607	–	0.267	1.000	0
14	0.684	–	0.428	1.000	0

Table 3. Retention times (t); retention factors (k'), separation factors (α) and resolution factors (R_S) for HPLC of 14 oxiranes on a CHIRAL OJ stationary phase; mobile phase: heptane/iso-propanol (70/30), flow rate: 0.5 mL/min, $t_0 = 1.212$

Compounds	t_1	t_2	k'	α	R_S
1	8.531	–	6.039	1.000	0
2	8.917	–	6.357	1.000	0
3	7.361	8.435	5.517	1.175	0.826
4	9.918	11.557	7.631	1.189	1.261
5	5.616	–	3.634	1.000	0
6	6.213	–	4.126	1.000	0
7	13.358	–	10.021	1.000	0
8	8.430	9.812	6.526	1.191	1.256
9	3.873	–	2.196	1.000	0
10	3.300	3.870	1.958	1.237	1.425
11	17.233	21.217	23.031	1.242	0.890
12	1.498	–	0.236	1.000	0
13	1.424	–	0.175	1.000	0
14	1.920	–	0.584	1.000	0

Table 4. Retention times (t); retention factors (k'), separation factors (α) and resolution factors (R_S) for HPLC of 14 oxiranes on a CHIRAL OD stationary phase; mobile phase: heptane/iso-propanol (90/10), flow rate: 0.5 mL/min, $t_0 = 1.304$

Compounds	t_1	t_2	k'	α	R_S
1	12.036	13.427	8.763	1.130	1.546
2	4.408	4.611	2.458	1.065	0.677
3	2.518	–	0.915	1.000	0
4	6.519	6.792	4.104	1.052	0.546
5	4.592	–	2.521	1.000	0
6	7.019	–	4.383	1.000	0
7	6.444	7.100	4.150	1.128	0.729
8	2.633	2.901	1.104	1.203	1.072
9	2.004	–	0.537	1.000	0
10	2.290	–	0.756	1.000	0
11	5.375*	6.119	3.370	1.183	0.827
12	1.587	–	0.207	1.000	0
13	1.534	–	0.106	1.000	0
14	1.728	–	0.314	1.000	0

*mobile phase: 70/30 *n*-heptane/iso-propanol.

Table 5. Retention times (t); retention factors (k'), separation factors (α) and resolution factors (R_S) for HPLC of 14 oxiranes on a CHIRAL OG stationary phase; mobile phase: heptane/iso-propanol (70/30), flow rate: 0.5 mL/min, $t_0 = 1.151$

Compounds	t_1	t_2	k'	α	R_S
1	12.852	–	10.166	1.000	0
2	5.848	–	4.081	1.000	0
3	2.202	–	0.913	1.000	0
4	9.337	11.043	7.853	1.208	1.706
5	7.230	8.815	5.970	1.261	1.761
6	8.905	10.155	7.280	1.161	1.250
7	3.547	–	2.082	1.000	0
8	2.737	2.882	1.441	1.091	0.725
9	1.826	–	0.586	1.000	0
10	2.503	–	1.175	1.000	0
11	2.417	–	1.100	1.000	0
12	1.749	–	0.520	1.000	0
13	1.536	–	0.334	1.000	0
14	1.680	–	0.460	1.000	0

where t_R is the peak retention time and t_0 is the column dead time. k' is the average retention factor for two peaks. The separation factor α is given as in Equation (2)

$$\alpha = k_2/k_1 \tag{2}$$

where k_2 and k_1 are the capacity factors for the second and first and eluted peaks, respectively The resolution factor R_s is defined as in Equation (3)

$$R_s = 2(t_2 - t_1)/(w_1 + w_2) \tag{3}$$

where, t_1 and t_2 are the retention times of the first and second eluted peaks, respectively, and w_1 and w_2 are their baseline bandwidths. R_s is used to describe the degree of separation between the two enantiomeric peaks.

In Figure 2, 14 racemic oxiranes were tested for their resolution using 5 different polysaccharide based CSPs. While linear cellulose derivatives form the basis of most polysaccharide stationary phases, helical amylose derivatives can provide widely different selectivities. The tris(3,5-dimethylphenylcarbamate) derivative of amylose (Chiralpak AD) often exhibits the highest enantioselectivity in this class of CSPs. This phase was successful in the resolution of 8 out of 14 racemic oxiranes tested in this study. It is interesting to note that the corresponding cellulose derivative, cellulose tris(3,5-dimethylphenyl carbamate) (Chiralcel OD)

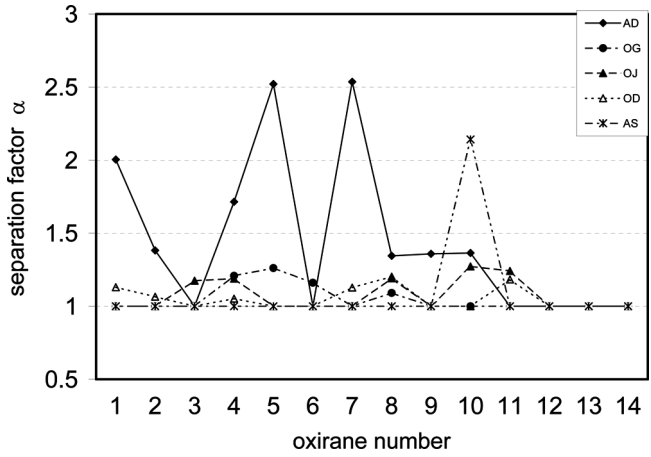


Figure 2. Enantiomeric separation of oxiranes **1** to **14** on 5 CSPs by HPLC.

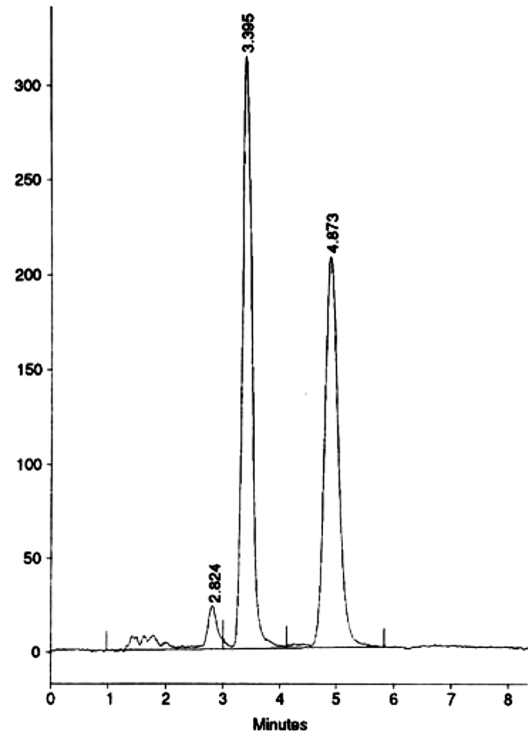


Figure 3. Chromatogram of compound No.4 with CHIRALCEL OD, with 30% of iso-propanol in haptane as mobile phase.

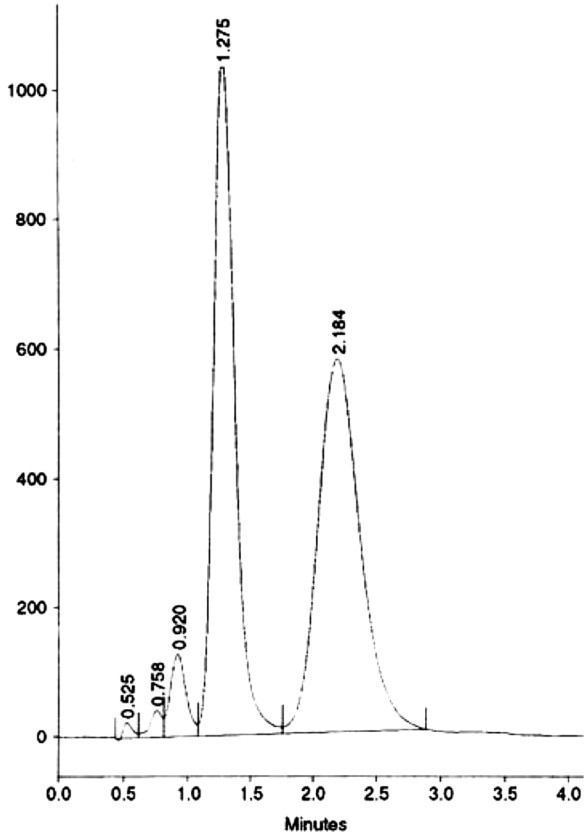


Figure 4. Chromatogram of compound No.10 with CHIRALPAK AS, with 10% of iso-propanol in haptane as mobile phase.

proved to be the second best phase, as it successfully separated 6 racemic oxirane compounds. The separation capability of these five stationary phases is in the order: Chiralpak AD > Chiralcel OD > Chiralcel OJ > Chiralcel OG > Chiralpak AS. Representative chromatograms for the enantiomeric separation of these oxirane derivatives are shown in Figures 3–7.

Comparison of Enantiomeric Separations on Five Different Polysaccharide based CSPs

From the chiral separation results, Chiralpak AD appears to be quite versatile, being successful to resolve 8 out of 11 oxiranes derivatives possessing an aromatic π-system, however no resolution was achieved with the oxirane derivatives possessing no π-aromatic system. The other amylose

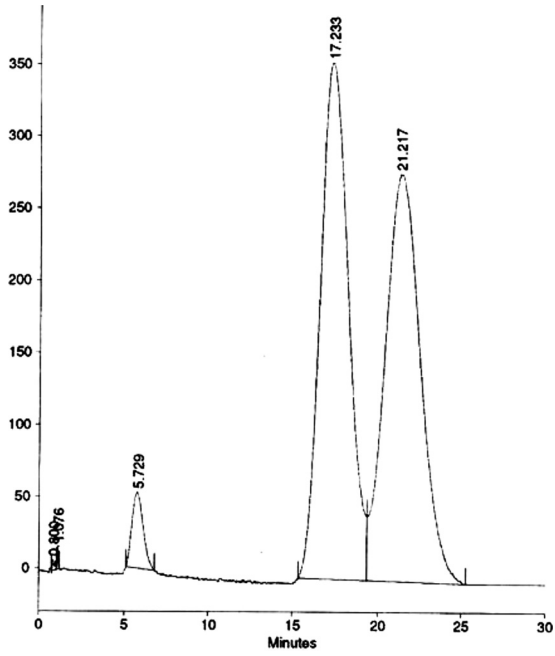


Figure 5. Chromatogram of compound No.11 with CHIRALCEL OJ, with 30% of iso-propanol in haptane as mobile phase.

phase Chiralpak AS, however, merely allows the separation of compound 10, probably due to its unique rod like molecular structure. In an analogy to Chiralcel AD, the similar cellulose derivative Chiral OD is also versatile, but not as effective; the separation factors (α) are much smaller, and compound 10, *inter alia*, is not separated at all. Chiralcel OG is also of limited applicability, but the selectivity is entirely different from Chiralcel OD. The methyl substituents play an important role in chiral recognition, with a mostly steric effect for the 3,5-dimethylphenylcarbamate (Chiralcel OD) and an electronic +M-effect for the 4-methyl derivative (OG). Likewise, 4-methyl-benzoate Chiralcel OJ is not very effective; however, the selectivity is again quite different from Chiralcel OG. Notably, the rod like compound 10 is effectively separated on Chiral AS.

Influence of the Analyte Structure on the Enantiomer Separation

The molecular structure of the analytes will also influence the enantiomeric separation and retention behaviour. The 14 analytes are divided into two groups, one where the π aromatic ring system (compounds 1 to 11 in Figure 1), and the other where the π aromatic ring system is

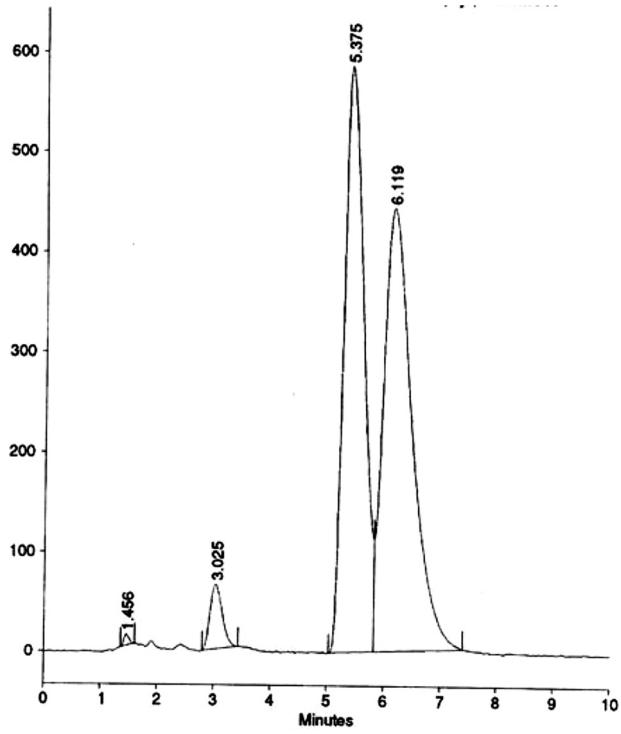


Figure 6. Chromatogram of compound No.11 with CHIRALCEL OD, with 30% of iso-propanol in haptane as mobile phase.

absent (compounds 12–14). Tables 1 to 5 indicate that compounds 12–14, which lack the π aromatic group could not be resolved, and they also have a rather short retention time. Hence, there are no sufficient interactions between these analytes and this type of CSPs. None of the 14 oxiranes can be separated on all five columns used in this study, but compounds 4 and 8 can be separated on four of them, while compound 10 can be separated on three different CSPs. Compound 1 has a strong retention due to the high polarity of this molecule, despite the lack of an aryl group. Compounds 12, 13, and 14, which are lacking the aromatic ring system and the carbonyl groups as well, show weak interactions with any of the five CSPs. However, the enantiomeric separation of 1 is not as good as some of the oxiranes bearing an aryl group. The compounds 3 and 11 do have aryl groups, but they do not bind very strongly to the stationary phases. In compound 6, the aryl groups are away from the stereogenic center, so it shows only a small enantioselectivity.

We conclude that dipole interactions have a strong impact on the retention mechanism, and extended π aromatic systems are mandatory.

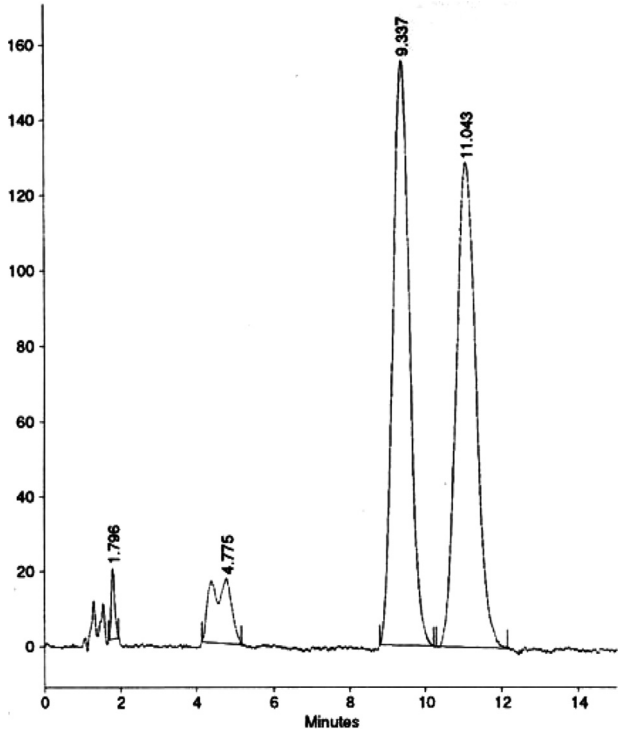


Figure 7. Chromatogram of compound No.4 with CHIRALCEL OG, with 30% of iso-ropanol in haptane as mobile phase.

The spatial arrangement of the substituent groups around the analyte stereogenic center plays an important role in enantiomeric separations. The closer a group is to the chiral center, the more likely is chiral recognition and enantioselectivity achieved. However, if there are no groups in the analyte that could bind to the stationary phase, the enantiomers are not likely to be separated.

CONCLUSIONS

Racemic oxirane compounds with different chemical structures possessing antitumor activity can be separated on several polysaccharide based chiral stationary phases by HPLC. The chemical structure of the analytes did play a role in the enantiomeric separation and retention behaviour. The dipole interactions, as well as the π aromatic system, have an impact on the chiral recognition mechanisms. The spatial arrangement of the substituent groups around the analyte stereogenic center plays an

important role in enantiomeric separations. The closer these functional groups are to the stereogenic center, the more likely is chiral recognition and enantioselectivity achieved.

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