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Application of Polysaccharide-Based Columns for the Separation of Racemic Mixtures Obtained Based on (R,S)-2-Octanol by Reversed-Phase Chiral Liquid Chromatography

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Abstract: The polysaccharide-based chiral stationary phase (CSP) columns for high-performance liquid chromatography (HPLC) were applied in the separation of liquid crystalline racemic mixtures obtained based on (R,S)-2-octanol and were found to be highly effective in recognizing isomers of minor structural differences. The mobile phase (MP), which consists of acetonitrile (ACN)/water (H_2O) at different volume ratios, was used. All measurements were made at room temperature. The order of the elution was also determined. The chromatographic parameters such as resolution - (Rs), selectivity - (α), and the number of theoretical plates, i.e., column efficiency - (N), were determined. The polysaccharide-based CSP columns have unique advantages in separation technology, and this study has shown the potential usefulness of the CSP columns in separating liquid crystalline racemic mixtures belonging to the same homologous series.

Keywords: racemates; enantiomers; liquid crystals; amylose derivative; cellulose derivative; liquid chromatography

1. Introduction

Racemic mixtures (racemates) contain optically active compounds called enantiomers; a racemic mixture is a 50:50 mixture of two enantiomers. There are right-handed enantiomers, which can rotate the plane of polarized light by a specific angle to the right, and left-handed enantiomers, which can rotate polarized light by the same angle to the left. Racemates show no optical rotation because the (+) rotation from one enantiomer cancels the (−) rotation from the other exactly [1–3]. Enantiomers generally have identical physical properties such as melting point, boiling point, infrared absorptions, and NMR spectra. It is important to realize, however, that while the melting point of one enantiomer will be identical to that of the other, the melting point of a mixture of the two enantiomers may differ.

Modern developments in science and technology have led to the chemical synthesis of many racemic mixtures, of which usually only the enantiomer with the desired properties is used, as was the case with thalidomide (Figure 1). Thalidomide was supplied as a racemic mixture of two enantiomers; the (R) enantiomer had the desired sedative effect, while the (S) enantiomer had embryotoxic and teratogenic effects [4–7]. This has resulted in enormous progress in the field of separation of the racemic mixtures. Among all chromatographic techniques, liquid chromatography has achieved particular development, enabling the analysis of samples in a few minutes.

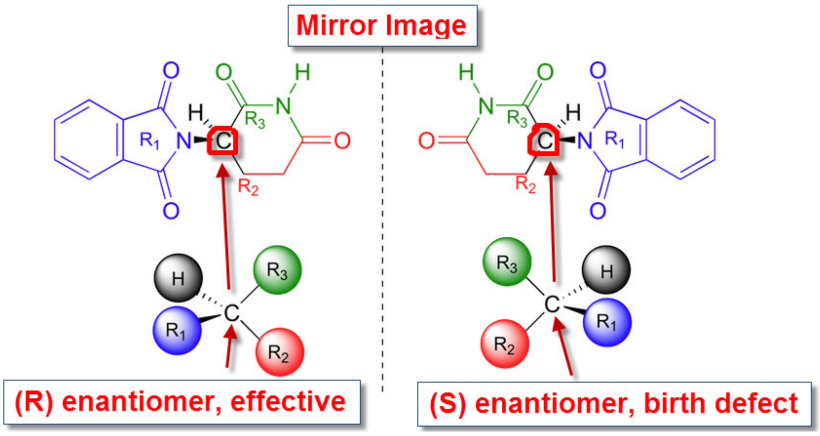

Figure 1. Properties of thalidomide enantiomers.

Liquid crystalline compounds and mixtures [8–10] can also be analyzed using high-performance liquid chromatography - HPLC. This technique is a commonly used analytical method. This is because it can be used for virtually any analyte or class of analytes, regardless of their properties. It allows you to perform analyses that would be difficult or impossible to perform using other types of chromatography. HPLC can achieve excellent resolution at high speed and sensitivity for separating high molecular weight polar compounds [11–20].

Chiral stationary phases (CSPs) are used for direct separation of enantiomers. Due to the principle of operation and their structure, the following can be distinguished [21]:

- polysaccharides,
- Pirkle-type phases,
- cyclodextrins,
- crown ethers,
- ligand exchange phases,
- ion-exchange phases,
- protein phases,
- macrocyclic antibiotics.

Polysaccharides are compounds widely found in nature. These are macromolecular compounds composed of simple sugars connected by glycosidic bonds. There are compounds containing several units, such as oligosaccharides, the simplest of which are disaccharides and polysaccharides. Polysaccharides include, among others, starch, cellulose, and glycogen. Polysaccharides play a role in living organisms with various functions: glycogen, starch, and inulin act as reserve substances; heparin prevents blood clotting; cellulose is the basic material of plant tissue; chitin is a compound occurring in the external skeletons of arthropods [22]. Polysaccharides also have an essential feature - they are optically active. For this reason, they are also used as stationary phases in the separation of mixtures of optically active compounds, and the most frequently used are derivatives of amylose and cellulose [16,20,23–30]. Polysaccharide derivatives embedded in silica gel show a sufficiently high recognition of chiral compounds, but unfortunately, they are only compatible with a limited amount solvents used as mobile phases, those that do not dissolve the stationary phase, such as hydrocarbon solvents and alcohols, acetonitrile and their aqueous solutions. This limitation in the selection of eluent components is a serious disadvantage because the efficiency depends on the selection of components and the composition of the eluent separation of enantiomers. For this reason, it was decided that the chemical immobilization of polysaccharides on solid support would be the only good solution to expand the range of applicability of various types of solvents as components of mobile phases. The first immobilization of polysaccharide derivatives on silica gel was made using diisocyanate and has been checked by the effectiveness of other methods, such as radical polymerization, photoirradiation, polymerization catalyzed by enzymes, and others. This allowed using many other solvents, such as chloroform, dichloromethane, and tetrahydrofuran, as mobile phases of HPLC. The application of these solvents, in many cases, not only increased the efficiency of separating enantiomers but also allowed for obtaining a different selectivity profile for some racemic mixtures [31–33].

The choice of mobile phase significantly impacts the selectivity of the chromatographic system. The mobile phase is selected for the stationary phase, and the substance to be separated, depending on its molecular structure. The solubility of the substances separated in the eluent is important. The most commonly used solvents in the normal phase system are hexane, cyclohexane, ethyl acetate, and chloroform. Alcohols and ethers are used less frequently [14]. Aqueous solutions of acetonitrile and methanol are often used in the reversed-phase (RP) system. As in the case of the normal phase (NP) system, the selection of eluent components depends on the solubility and physical properties of the substance to be separated. In a reversed-phase system, the choice of solvents is quite limited [30,34]. Depending on the composition of the mobile phase, two different modes are generally applicable. If the makeup of the mobile phase remains constant during the separation process, the HPLC system is defined as an isocratic elution system. When the composition of the mobile phase is changed during separation, the HPLC system is defined as a gradient elution system [35,36].

Chiral stationary phases use various types of chiral recognition mechanisms, which enable the separation of enantiomers of chemical compounds belonging to different classes. Some of the available CSPs are universal, making them very popular. Such phases include cellulose and amylose derivatives, which were used in the research described in the work, the aim of which was to optimize the separation of nineteen racemic mixtures obtained based on (R,S)-2-octanol into optically active enantiomers using chiral liquid chromatography on two chiral columns: ReproSil Chiral-MIG and ReproSil Chiral-MIC. These were two immobilized columns from Dr. Maisch [37] with different column packing materials. The mixtures were separated using the isocratic and the gradient elution. During the studies, the type of column, volume ratio of solvents, mobile phase flow rate, and the elution type were changed. The elution order of the enantiomers on these two chiral columns was also determined. Finding optimal separation conditions would avoid expensive syntheses of enantiomers of these racemic mixtures.

2. Results and Discussion

2.1. Racemic Mixtures

The studies concerned nineteen racemic mixtures differing in the oligomethylene chain's length and the benzene ring's substituents, see Figure 2.

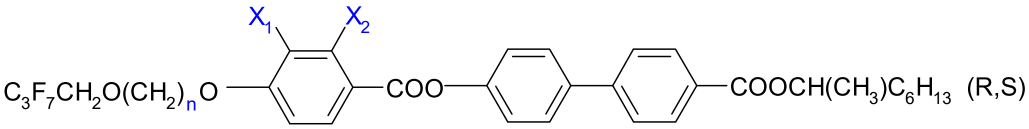

Figure 2. General formula of the racemic mixtures used in the studies, where: *n* - length of the oligomethylene chain (*n*= 2, 3, 5, 6, 7), and X1, X2 - substituents of the benzene ring (fluorine or hydrogen).

Depending on the number of methylene groups and the substitution of the benzene ring, each mixture was given an appropriate acronym (Table 1). These mixtures were synthesized as shown in Refs. [38,39]. The studied racemates were prepared by treating (R,S) 4′-(1-methylheptyloxycarbonyl)biphenol with benzoic acid chloride. The efficient preparation of achiral biphenol was described in Ref. [40]. The commercially available (R,S)-2-octanol with a purity of 99.5% was used. The studied racemates are liquid crystals with smectic phases (SmC$_A$ and/or SmC and/or SmA).

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Table 1. The acronyms of the racemic mixtures.

2.(HH) (R,S)	3.(HH) (R,S)	5.(HH) (R,S)	6.(HH) (R,S)	7.(HH) (R,S)
2.(HF) (R,S)	3.(HF) (R,S)	5.(HF) (R,S)	6.(HF) (R,S)	7.(HF) (R,S)
2.(FH) (R,S)	3.(FH) (R,S)	5.(FH) (R,S)	6.(FH) (R,S)	7.(FH) (R,S)
-	3.(FF) (R,S)	5.(FF) (R,S)	6.(FF) (R,S)	7.(FF) (R,S)

The initial stage of preparing samples for analysis was weighing approximately 1.5 mg of each racemic mixture. The next step in sample preparation was to dissolve each weighed sample in 2.5 mL acetonitrile. The next part of the analysis was selecting the optimal parameters for separating racemic mixtures into enantiomers. For this purpose, the following changes were made during the studies:

- chromatographic columns,
- volume ratio of solvents,
- flow rate of the mobile phase,
- the type of elution.

Samples of non-equimolar mixtures - (R,S) + (S) or (R,S) + (R) were also prepared to determine the elution order. Approximately 1.5 mg of the sample was weighed and dissolved in 2.5 mL acetonitrile.

2.2. Chiral HPLC Separation

The chiral stationary phases (CSPs) made by covalent immobilization of amylose tris(3-chloro-5-methylphenylcarbamate) on silica gel (ReproSil Chiral-MIG) and cellulose tris(3,5-dichlorophenylcarbamate) on silica gel (ReproSil Chiral-MIC) were used, see Figure 3.

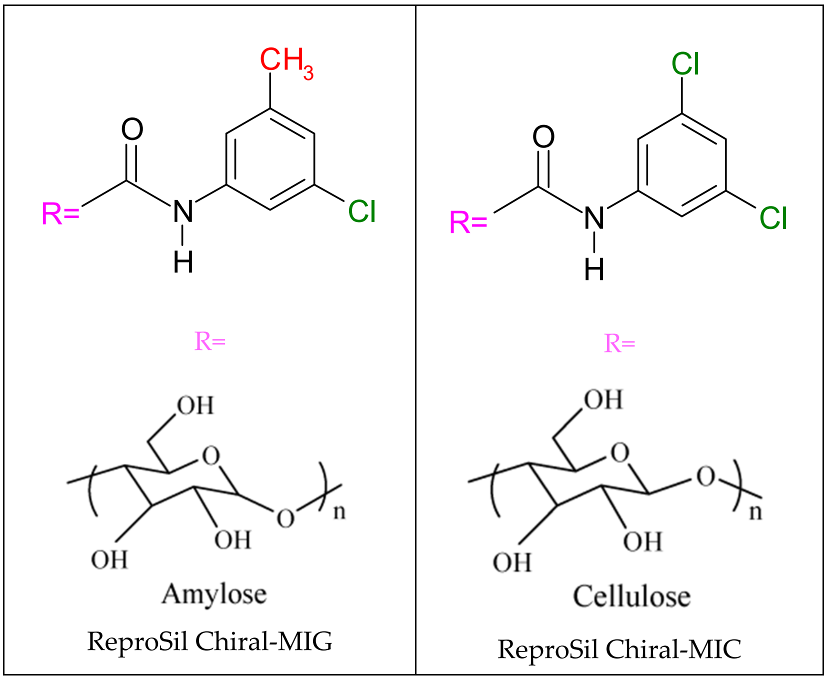

Figure 3. The columns used in the separation.

The columns had a particle size of 5 μm, dimensions of 250 mm × 4.6 mm i.d, and pore size of 1000 Å (Dr. Maisch, Germany). The Shimadzu LC-20AP HPLC system (Kyoto, Japan) consisting of a binary solvent delivery pump, an autosampler (SIL-10AP), a communications bus module (CBM-20A), a diode array detector (SPD-M20A) and a fraction collector (FRC-10A) were used for the separation and detection of analytes. Data acquisition was performed by Shimadzu software. The injection volume of samples was 10 or 15 μL. The measurements were carried out at room temperature. The mobile phase flow rate was 0.3, 0.5, or 1 mL·min^{-1}, and the detection wavelength was 254 nm.

The mobile phase consisted of acetonitrile/water at different volume ratios. Elution was performed in the isocratic mode and the gradient mode. The sample concentrations were 0.5-0.6 mg·mL^{-1}. The samples were dissolved in ACN. All solvents were used as purchased (ACN - POCH S.A.). In addition, ultrapure water was used.

2.3. ReproSil Chiral-MIG Column

All studied racemic mixtures were separated using the isocratic elution (Table 2) and the gradient elution (Table 3).

Table 2. The analysis parameters on the ReproSil Chiral-MIG column in the isocratic elution.

No.	**v/v**		**Flow rate [mL·min^{-1}]**	**Injection volume [µl]**
	CAN	**H$_2$O**		
1.	99	1	1	15
2.	95	5	1	15
3.	90	10	1	15

Table 3. The analysis parameters on the ReproSil Chiral-MIG column in the gradient elution.

Decreasing Gradient			**Increasing-Decreasing Gradient**		
Time [min]	**ACN/H$_2$O (v/v)**	**Injection volume [µl]**	**Time [min]**	**ACN/H$_2$O (v/v)**	**Injection volume [µl]**
0.01	99/1	10	0.01	92/8	10
5	96/4		3	94/6	
10	93/7		6	96/4	
15	90/10		9	98/2	
20	STOP		12	95/5	
-	-		15	92/8	
-	-		20	STOP	

In the acetonitrile-water solvent system, with a volume ratio of 99:1, mixtures 2.(HF) (R,S) (Figure 4a) and 3.(FF) (R,S) were not separated. The mixtures 2.(HH) (R,S) (Figure 4b), 2.(FH) (R,S), 3.(HH) (R,S), 3.(HF) (R, S), 3.(FH) (R,S), 5.(FH) (R,S), 5.(FF) (R,S), 6.(FH) (R,S), 6.(FF) (R,S), 7.(FH) (R,S) and 7.(FF) (R,S) were partially separated, and all remaining mixtures were baseline-separated - Rs>1.5 (Figure 4c).

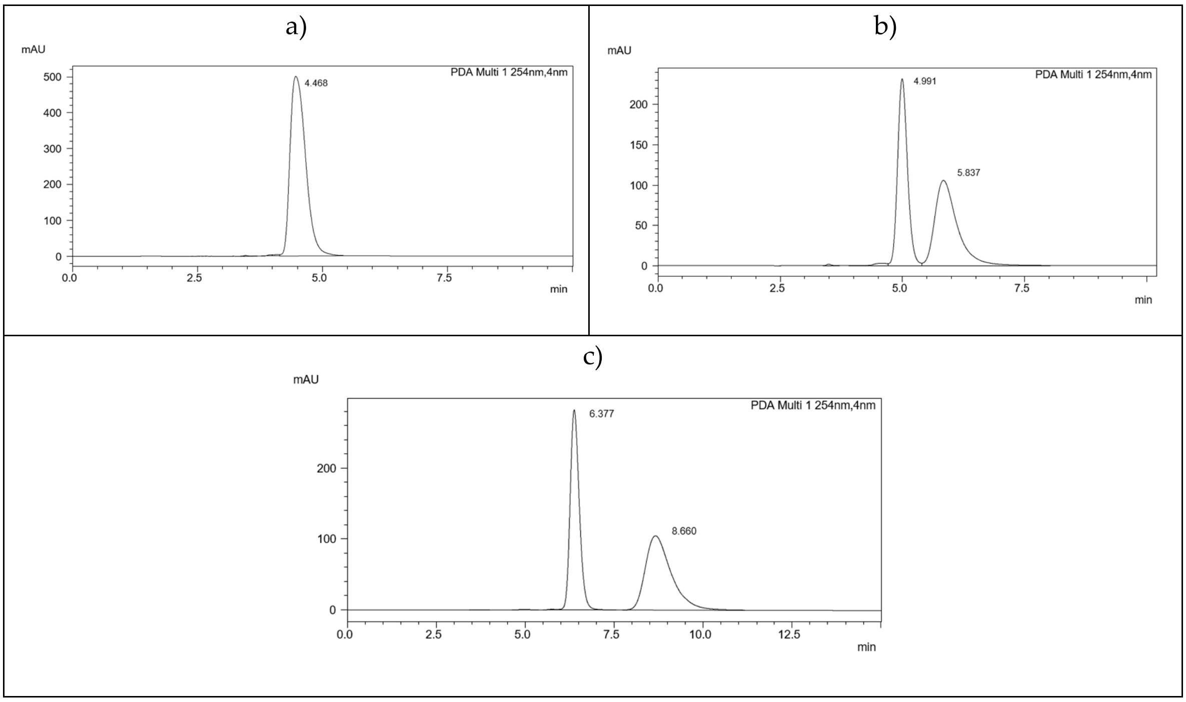

Figure 4. The chromatograms of enantioseparation of the racemic mixtures: a) 2.(HF) (R,S); b) 2.(HH) (R,S); c) 6.(HH) (R,S) obtained in the isocratic elution, the mobile phase: ACN/H_2O (99:1 v/v), the flow rate: 1 mL·min^{-1}.

All chromatographic parameters were calculated using the equations presented in Ref. [41]. The most crucial thing in HPLC is to obtain the optimum resolution in the minimum time. A resolution value of 1.5 or greater between two peaks will ensure that the sample components are well (baseline) separated to a degree at which the area or height of each peak may be accurately measured.

Figure 5 summarizes the resolution parameter values for the analyzed racemic mixtures. The highest value of this parameter was achieved by mixture 7.(HH) (R,S). It can also be noticed that mixtures with two hydrogen atoms at the benzene ring have higher resolution values than those with other substitutions. The lowest values of the resolution parameter were obtained for mixtures with two fluorine atoms on the benzene ring.

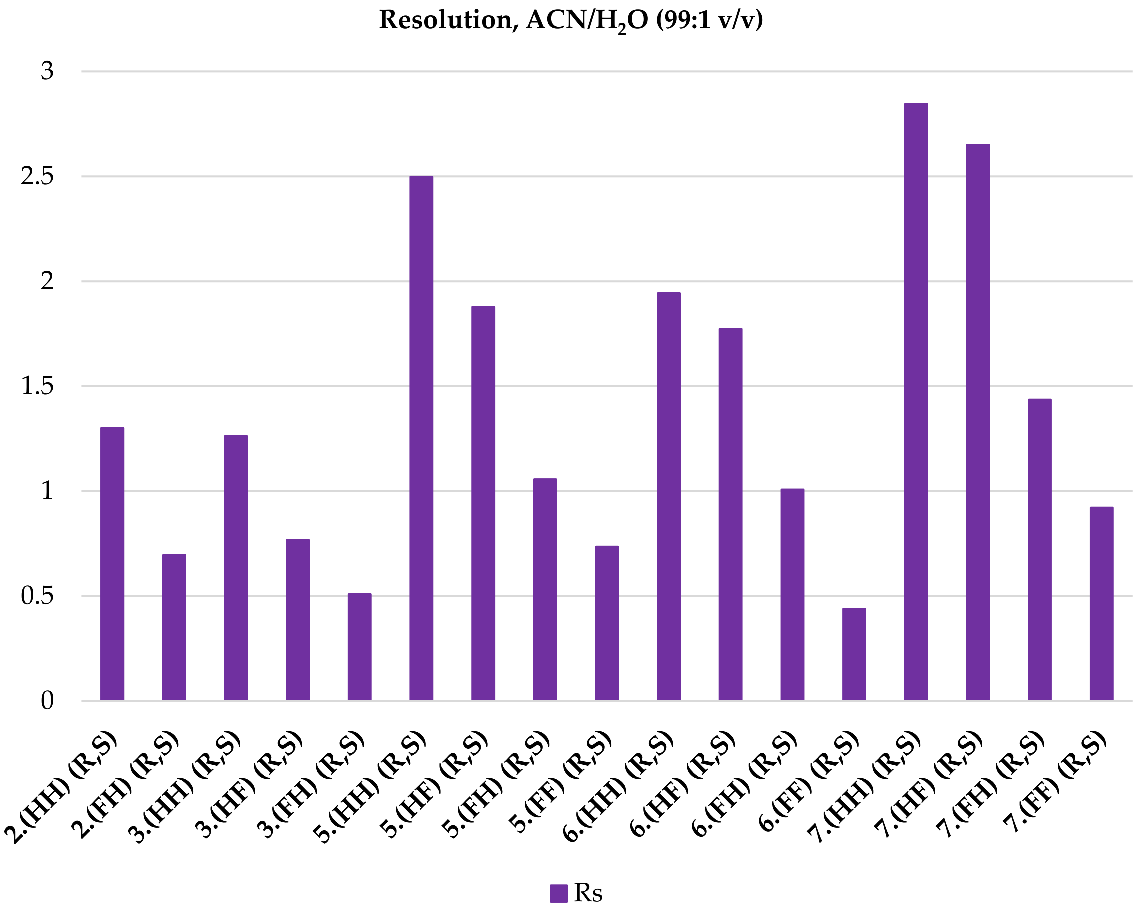

Figure 5. The resolution data obtained for the studied racemic mixtures on the MIG column in the isocratic elution, ACN/H_2O (99:1 v/v).

Figure 6 shows the retention times of the analyzed racemic mixtures. The mixtures with the highest resolution values showed the longest retention times. Retention times were increased with the length of the oligomethylene chain.

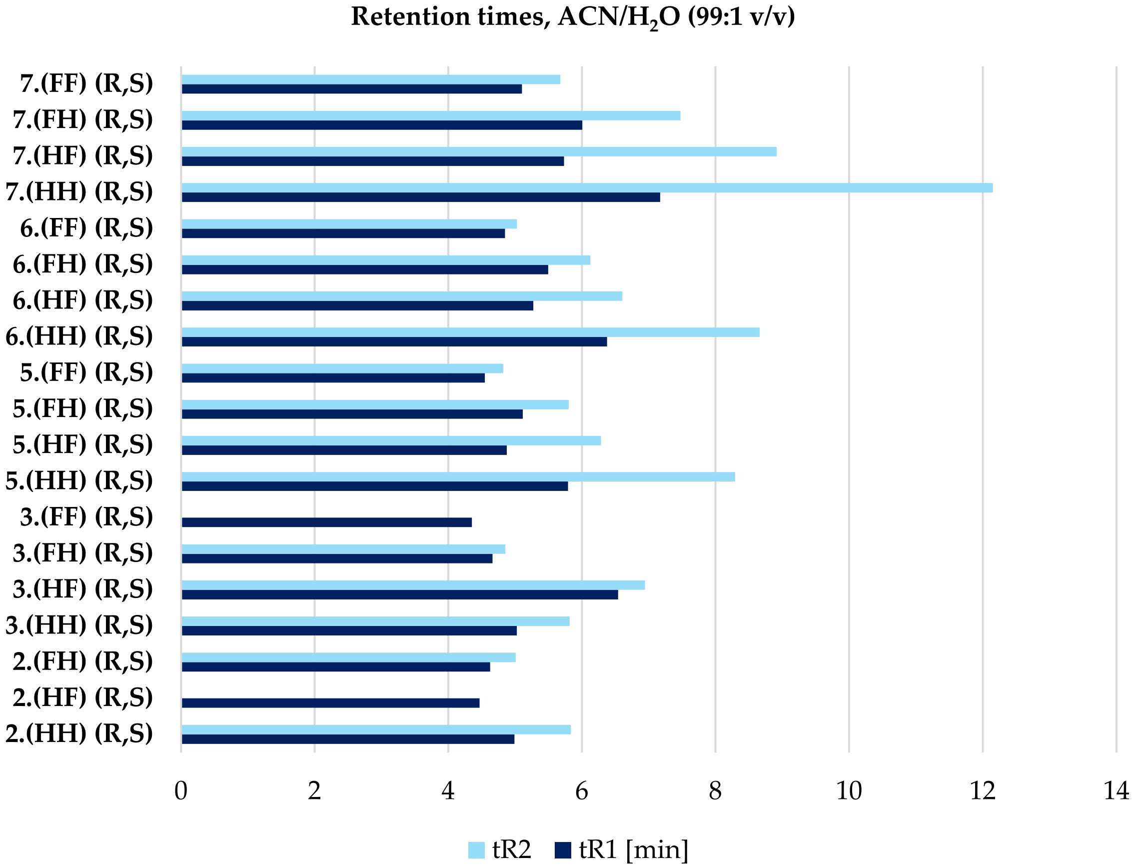

Figure 6. The retention times of the racemic mixtures on the MIG column in the isocratic elution, ACN/H_2O (99:1 v/v).

Analysis of racemic mixtures in non-equimolar ratios allowed the determination of the elution order of the enantiomers. The (S) enantiomer was first eluted in all mixtures, as shown in Figure 7 for the mixture 6.(FH) (R,S) + (S).

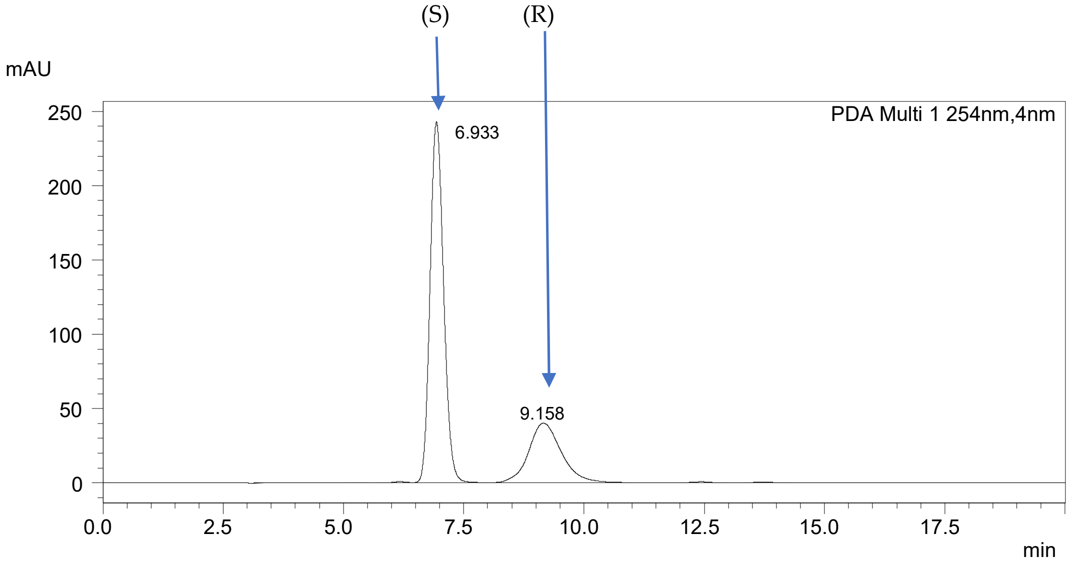

Figure 7. Chromatogram of the mixture 6.(FH) (R,S) + (S) obtained in the isocratic elution, ACN/H_2O (95:5 v/v), the flow rate 1 mL·min^{-1}.

In the Supplementary Materials, Figure S1 summarizes the values of the number of theoretical plates for the racemic mixtures that have been baseline-separated. The plate number (N) measures the HPLC column's peak dispersion, reflecting the column performance. Therefore, the more theoretical plates available within the column, the more equilibrations are possible and the better the quality of the separation (the column with a high number of plates gives narrower, more efficient peaks). Among the analyzed mixtures, the highest ability to produce narrow peaks was demonstrated by mixture 6.(HF) (R,S) (Figure S2), and the mixture 6.(HH) (R,S) had the lowest ability (Figure S3). Figure S4 summarizes the values of the selectivity parameter – α, i.e., the ability of a chromatographic system to "chemically" distinguish between sample components. The mixtures that separated and had the largest difference in retention times showed the highest selectivity value. The value of this parameter for mixtures that have not separated is 1. As the selectivity of the separation depends upon the chemistry of the analyte, mobile, and stationary phases, all of these factors may be altered to change or optimize the selectivity of the HPLC separation.

In the acetonitrile-water solvent system, with a volume ratio of 95:5, the mixture 3.(FF) (R,S) was not separated (see Figure S5 in the Supplementary Materials). The mixtures 2.(HF) (R,S) (see Figure S6), 3.(FH) (R,S) and 6.(FF) (R,S) were partially separated. The remaining mixtures were baseline-separated (see Figure S7). This system showed higher values of chromatographic parameters than the system with a volume ratio of 99:1. One mixture did not separate, while most of the racemic mixtures were baseline-separated.

Figure 8 summarizes the analyzed racemic mixtures' resolution parameter (Rs) values. As in the previous system, the highest value of this parameter was achieved by the mixture 7.(HH) (R,S). In this volume system, one can also notice the relationship that mixtures with two hydrogen atoms on the benzene ring have higher parameter values than other types of substitutions. The lowest values of the resolution parameter were obtained for mixtures with hydrogen and fluorine atoms at the benzene ring.

Figure 9 shows the retention times of the analyzed racemic mixtures. As in the previous system, the mixtures with the highest resolution values showed the longest retention times. Retention times were increased with the length of the oligomethylene chain.

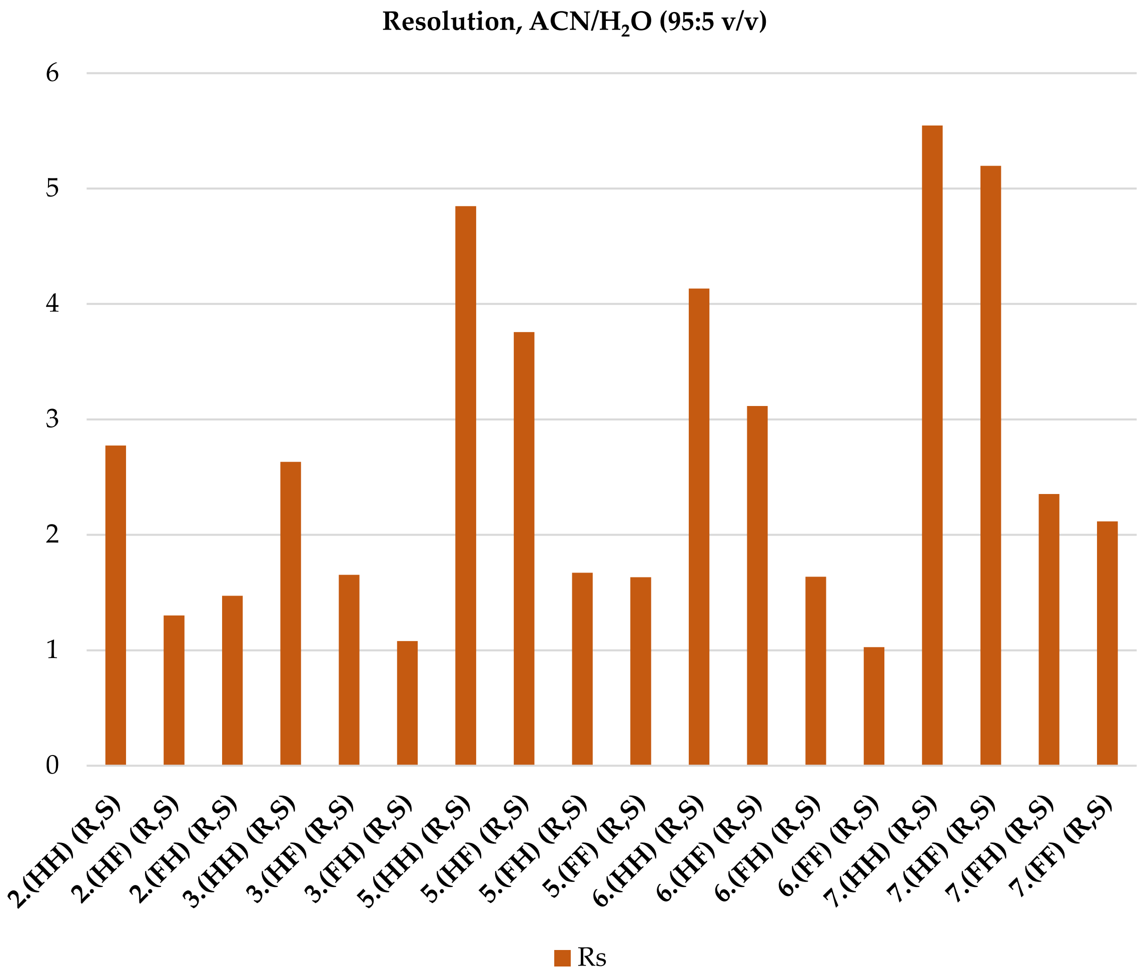

Figure 8. The resolution data obtained for the studied racemic mixtures on the MIG column in the isocratic elution, ACN/H_2O (95:5 v/v).

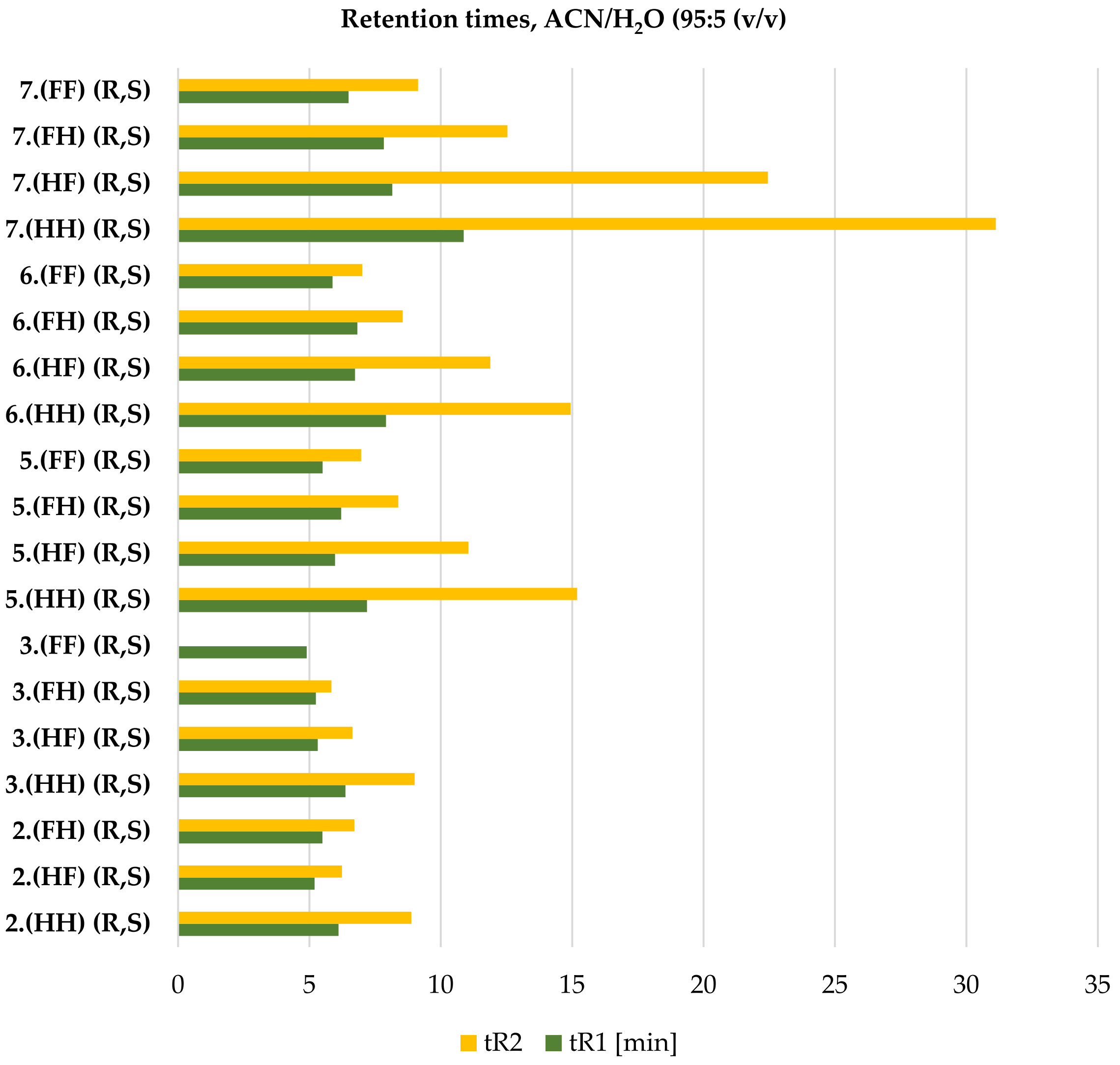

Figure 9. The retention times of the racemic mixtures on the MIG column in the isocratic elution, ACN/H_2O (95:5 v/v).

Figure S8 (in Supplementary Materials) summarizes the values of the number of theoretical plates for the racemic mixtures that have been baseline-separated. The highest ability to produce narrow peaks was demonstrated by mixture 3.(HH) (R,S) (Figure S9), and the lowest by mixture 7.(HH) (R,S) (Figure S10). Figure S11 summarizes the values of the selectivity parameter - α in the isocratic elution in a volume ratio of 95:5. The mixtures that separated and had the largest difference in retention times showed the highest selectivity value.

All racemic mixtures were separated in the acetonitrile-water solvent system in a volume ratio of 90:10. The mixtures 3.(HH) (R,S), 3.(FF) (R,S) and 5.(FH) (R,S) were partially separated. The remaining mixtures were baseline-separated. This system showed the highest values of chromatographic parameters of all three systems in the isocratic elution (Figures 10, S12, and S13).

Figure 10 summarizes the analyzed racemic mixtures' resolution parameter (Rs) values. Unlike the previous systems, the highest value of this parameter was achieved by mixture 6.(HH) (R,S). This system cannot be assigned a relationship that would characterize each type of benzene ring substitution. The highest resolution values are obtained for the mixtures with two hydrogen atoms on the benzene ring and those with hydrogen and fluorine atoms on the ring.

Figure 11 shows the retention times of the analyzed racemic mixtures. As in the previous systems, the mixtures with the highest resolution values showed the longest retention times.

Retention times were similar for most mixtures; only mixtures with six or seven methylene groups showed longer retention times.

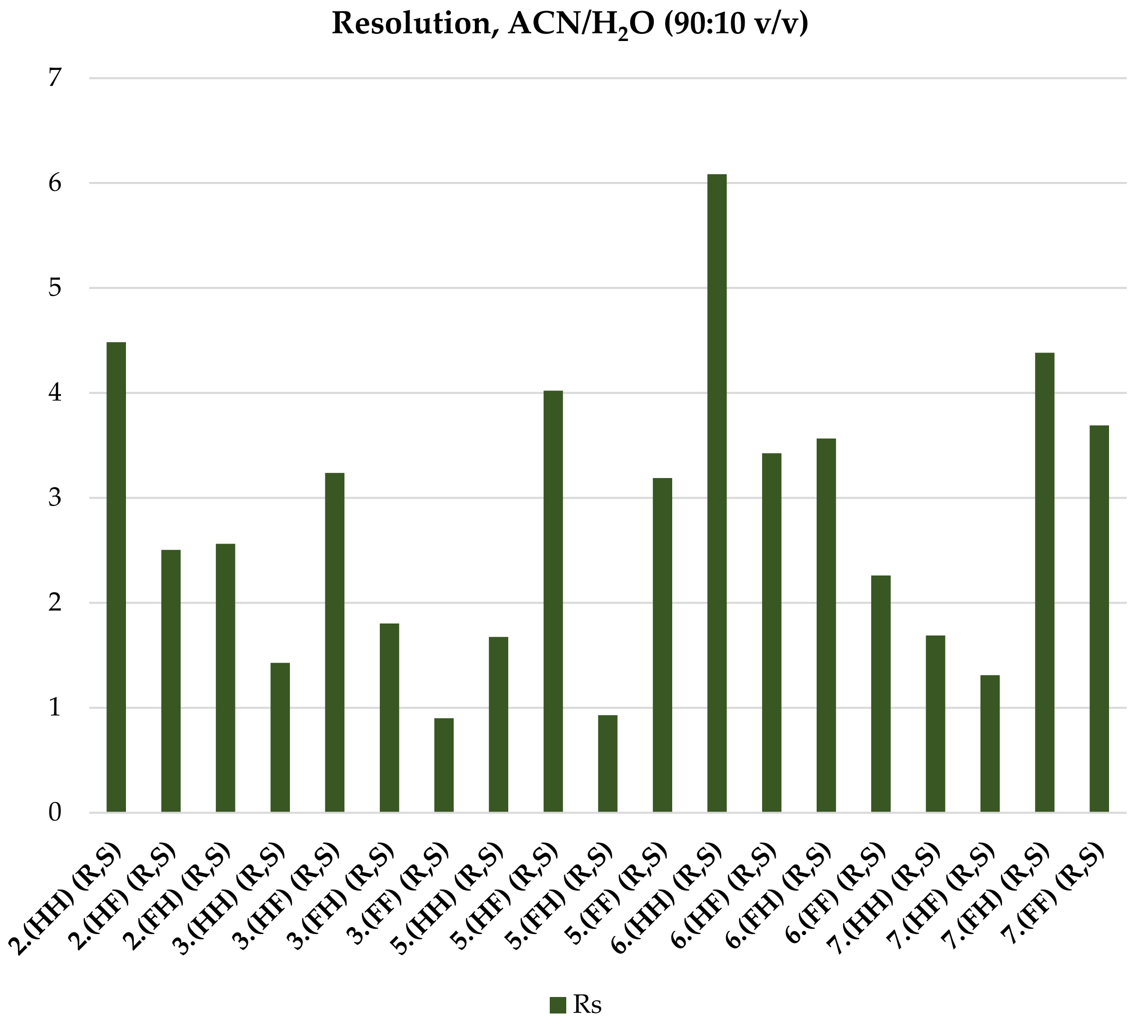

Figure 10. The resolution data obtained for the studied racemic mixtures on the MIG column in the isocratic elution, ACN/H_2O (90:10 v/v).

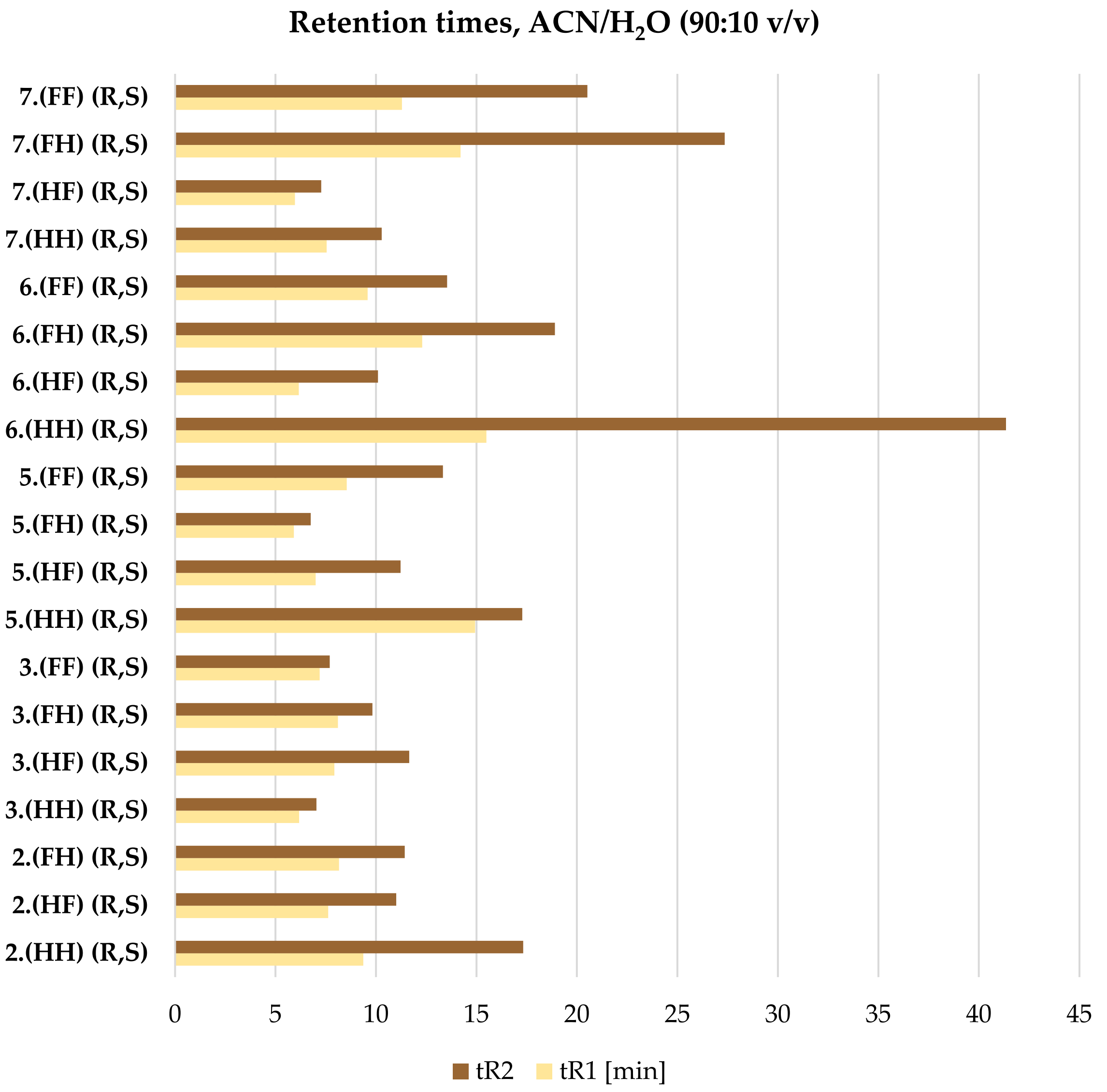

Figure 11. The retention times of the racemic mixtures on the MIG column in the isocratic elution, ACN/H_2O (90:10 v/v).

Figure S12 (in the Supplementary Materials) summarizes the values of the number of theoretical plates for the racemic mixtures that have been baseline-separated in a 90:10 volume system. The mixture 5.(HH) (R,S) had the highest ability to produce narrow peaks, while the lowest, as in the previous system, was 7.(HH) (R,S) (see Figure S13).

Figure S14 summarizes the values of the selectivity parameter - α in the isocratic elution in a volume ratio of 90:10. Mixture 6.(HH) (R,S) achieved the highest selectivity value, and mixture 3.(FF) (R,S) the lowest.

In the decreasing gradient, the mixtures 2.(HF) (R,S) and 3.(FF) (R,S) were not separated. The mixtures 2.(HH) (R,S), 2.(FH) (R,S), 3.(HH) (R,S), 3.(HF) (R, S), 3.(FH) (R,S), 5.(FH) (R,S), 5.(FF) (R,S), 6.(FH) (R,S), 6.(FF) (R,S), 7.(FH) (R,S) and 7.(FF) (R,S) were partially separated. The remaining mixtures were baseline-separated. This system showed much lower values of chromatographic parameters than previous isocratic elution systems. Most racemic mixtures did not separate or partially separate. Figure 12 summarizes the resolution parameter (Rs) values of the analyzed racemic mixtures in the gradient elution. Much lower resolution values characterize this system; only six racemic mixtures were baseline-separated out of seventeen separated mixtures. The highest resolution values for the mixtures with two hydrogen atoms on the benzene ring were found.

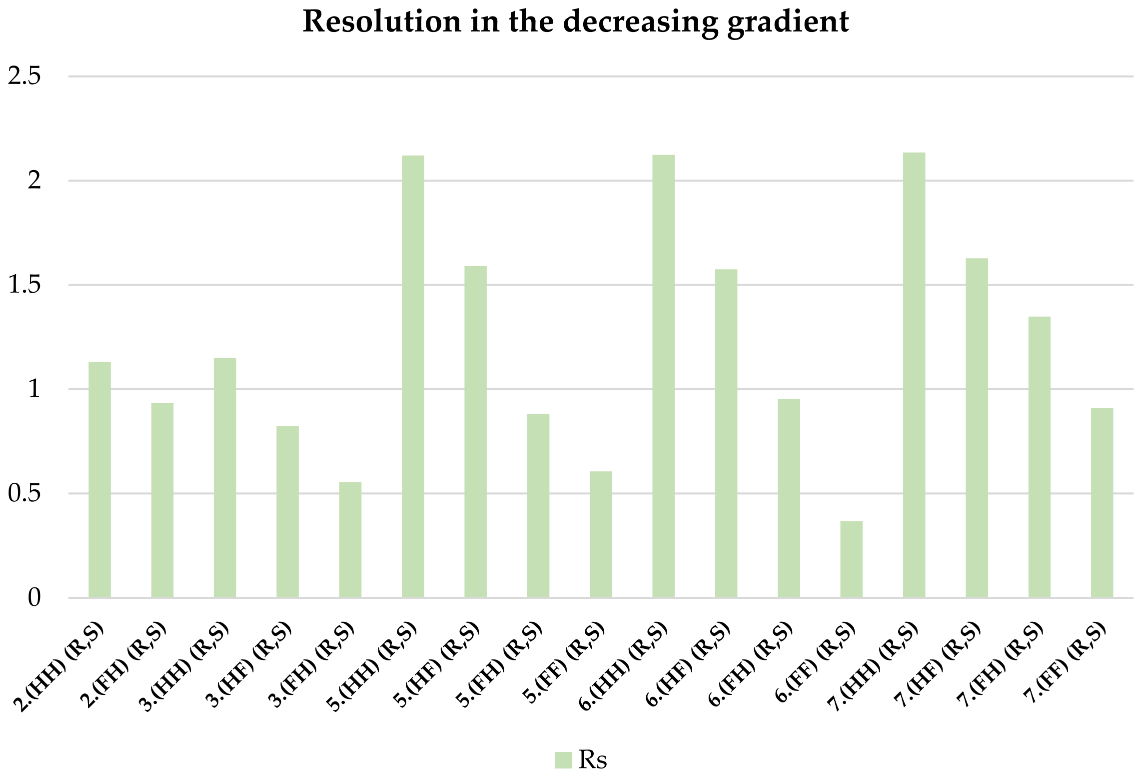

Figure 12. The resolution data obtained for the studied racemic mixtures on the MIG column in the decreasing gradient.

Figure 13 shows the retention times of the analyzed racemic mixtures in the decreasing gradient. As in the previous systems, the mixtures with the highest resolution values showed the longest retention times. As the oligomethylene chain lengthened, the retention times of the mixtures gradually increased, but this value was insignificant.

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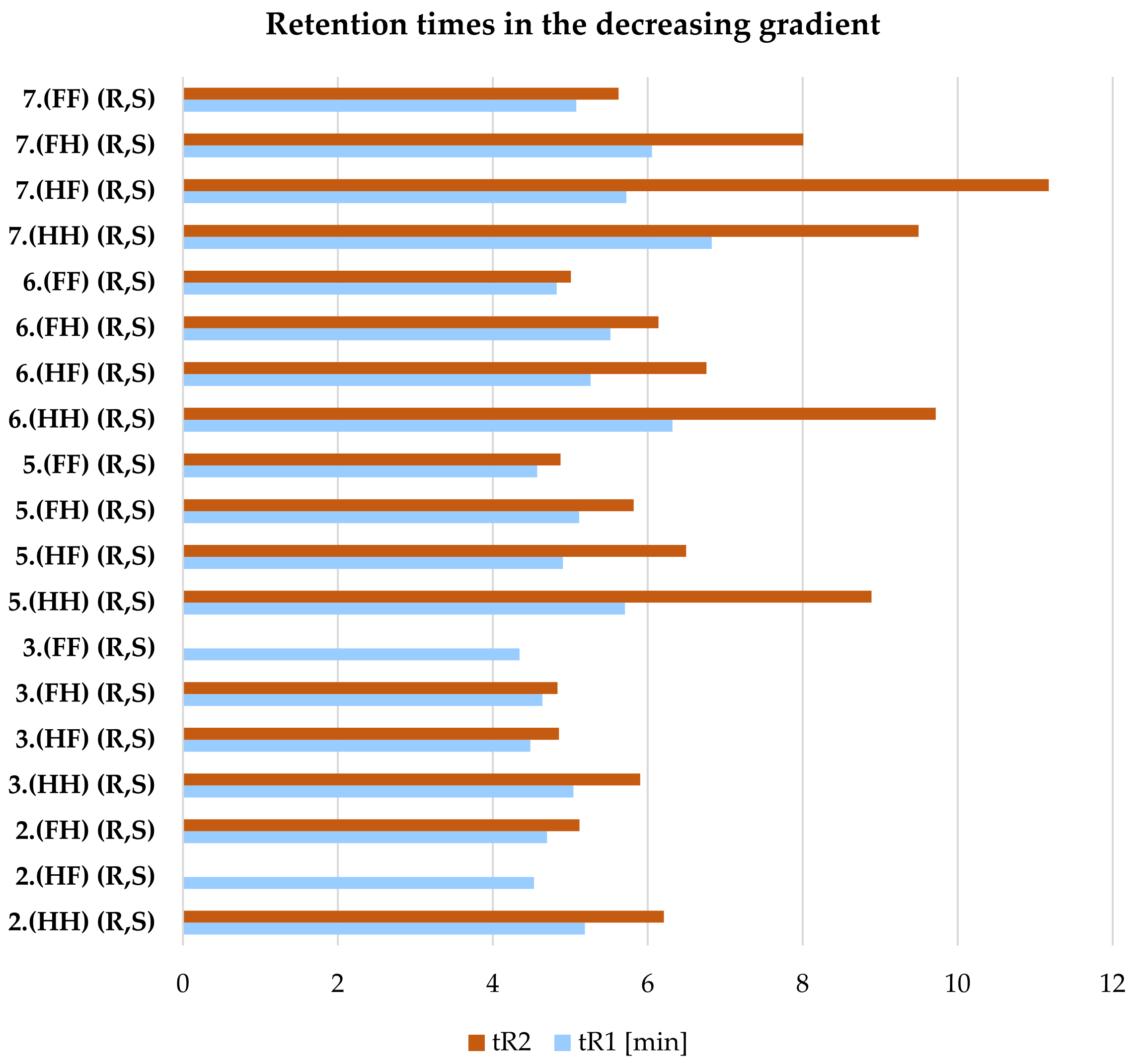


Figure 13. The retention times of the racemic mixtures on the MIG column in the decreasing gradient.

Figure S15 in the Supplementary Materials summarizes the values of the number of theoretical plates for the racemic mixtures baseline-separated in the decreasing gradient. The mixture 5.(HH) (R,S) (Figure S16) had the highest ability to produce narrow peaks, while the lowest was mixture 6.(HF) (R,S) (Figure S17). Figure S18 summarizes the values of the selectivity parameter in the decreasing gradient. The mixture 7.(HF) (R,S) achieved the highest selectivity value, and the mixtures 2.(HF) (R,S) and 3.(FF) (R,S) the lowest.

In the increasing-decreasing gradient, all racemic mixtures were separated. Mixtures 3.(FF) (R,S) and 7.(HF) (R,S) were partially separated. The remaining mixtures were baseline-separated. This system showed much higher values of chromatographic parameters than the decreasing gradient. It also had much higher parameter values than all other systems in the isocratic elution (see Figures 14–16).

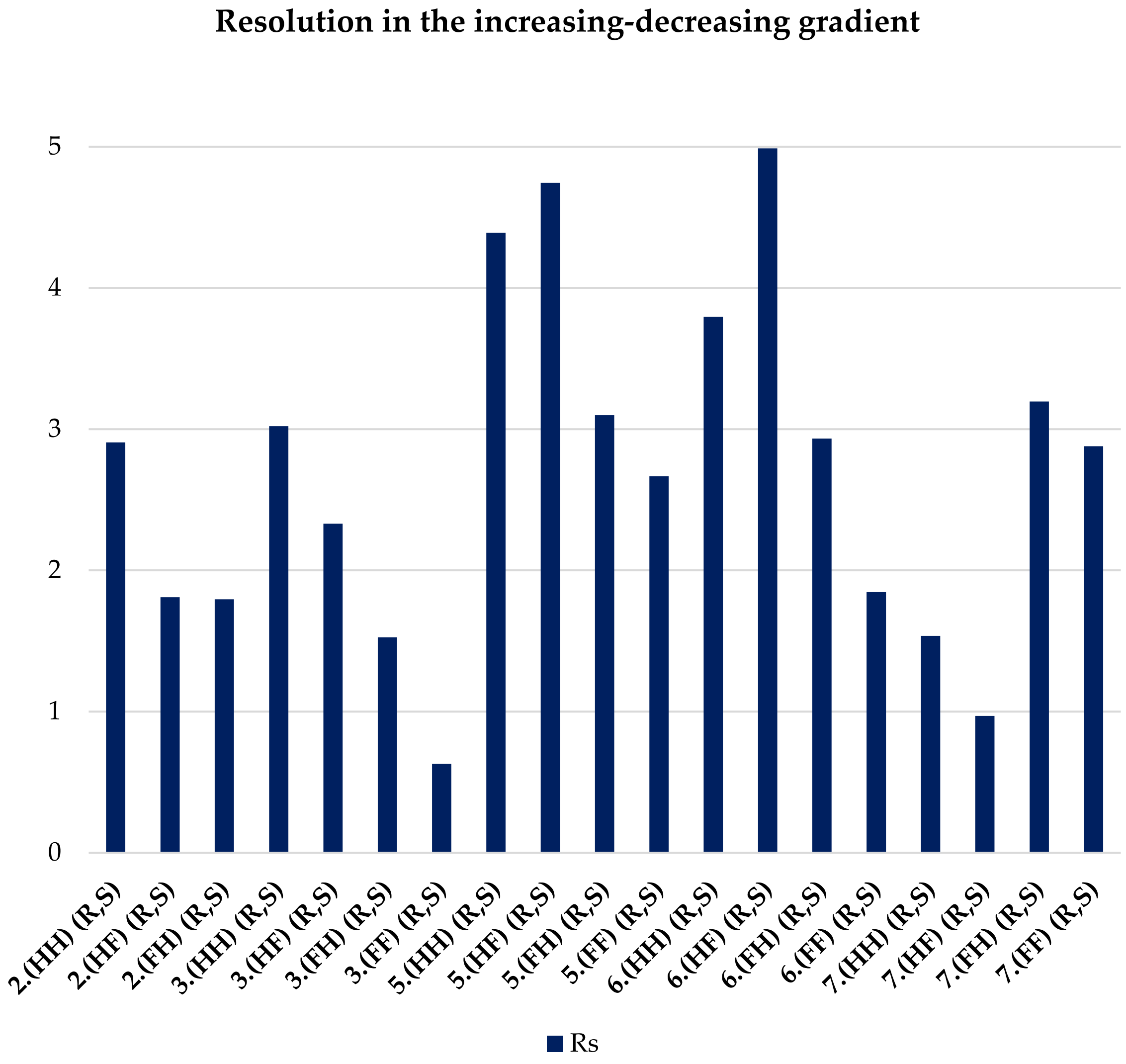


Figure 14. The resolution data obtained for the studied racemic mixtures on the MIG column in the increasing-decreasing gradient.

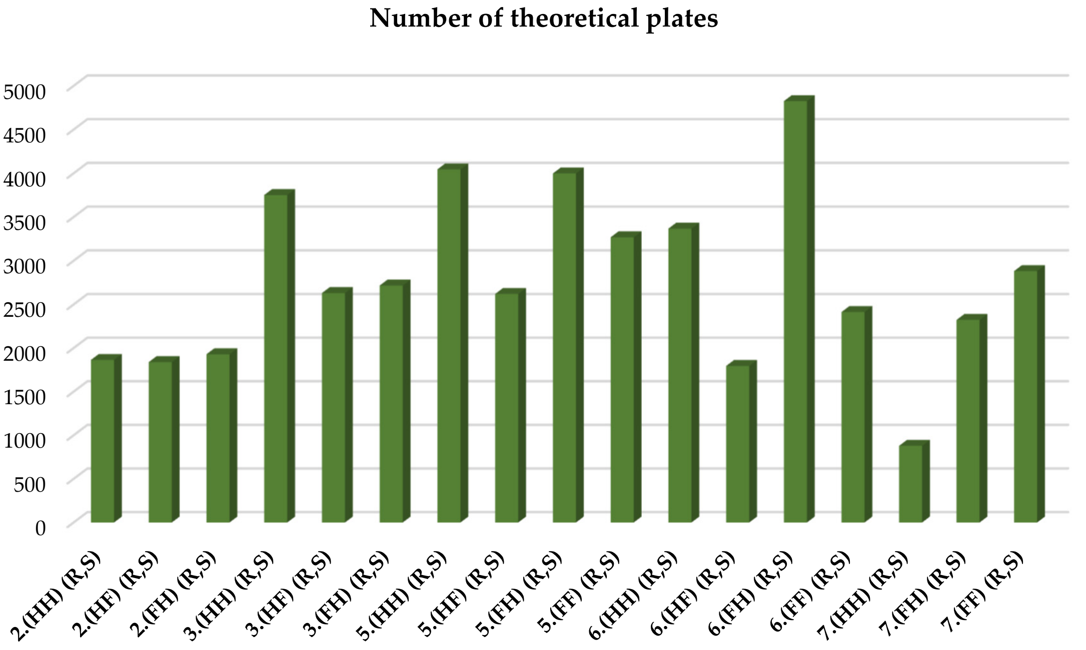


Figure 15. Theoretical plate values on the MIG column in the increasing-decreasing gradient, the flow rate 1 mL·min^{-1}.

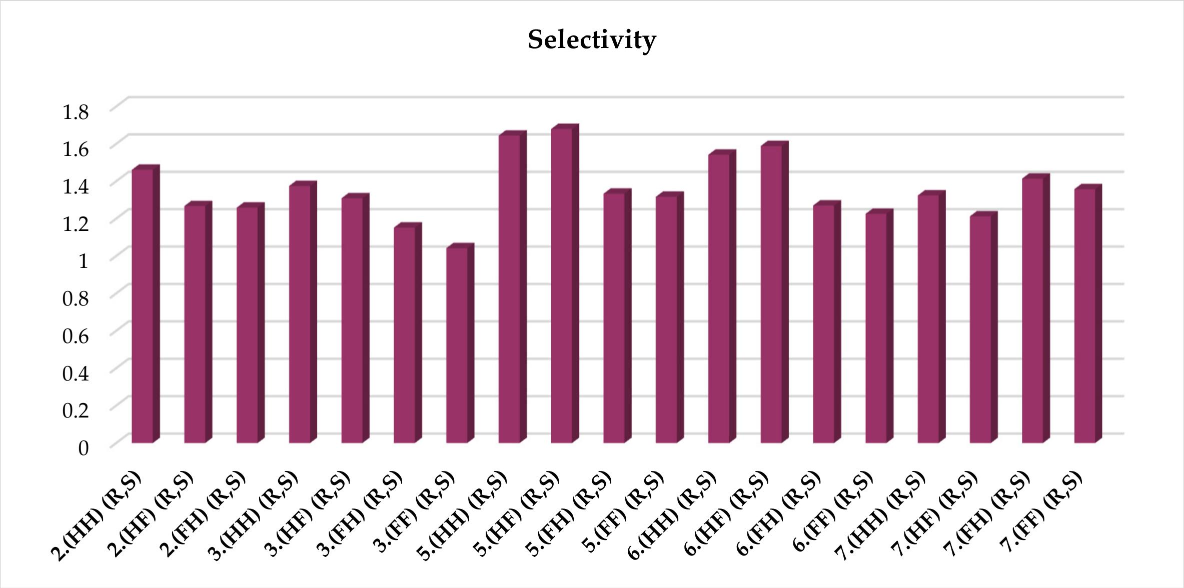


Figure 16. Selectivity values on the MIG column in the increasing-decreasing gradient, the flow rate 1 mL·min^{-1}.

The mixture 6.(FH) (R,S) had the highest ability to produce narrow peaks, while the lowest was mixture 7.(HH) (R,S) (Figure 60). The mixture 5.(HF) (R,S) achieved the highest selectivity value, and the mixture 3.(FF) (R,S) the lowest.

Figure 17 shows the retention times of the analyzed racemic mixtures in the increasing-decreasing gradient. As in the previous systems, the mixtures with the highest resolution values showed the longest retention times. The longest retention times were found for the racemic mixtures having two hydrogen atoms on the benzene ring.

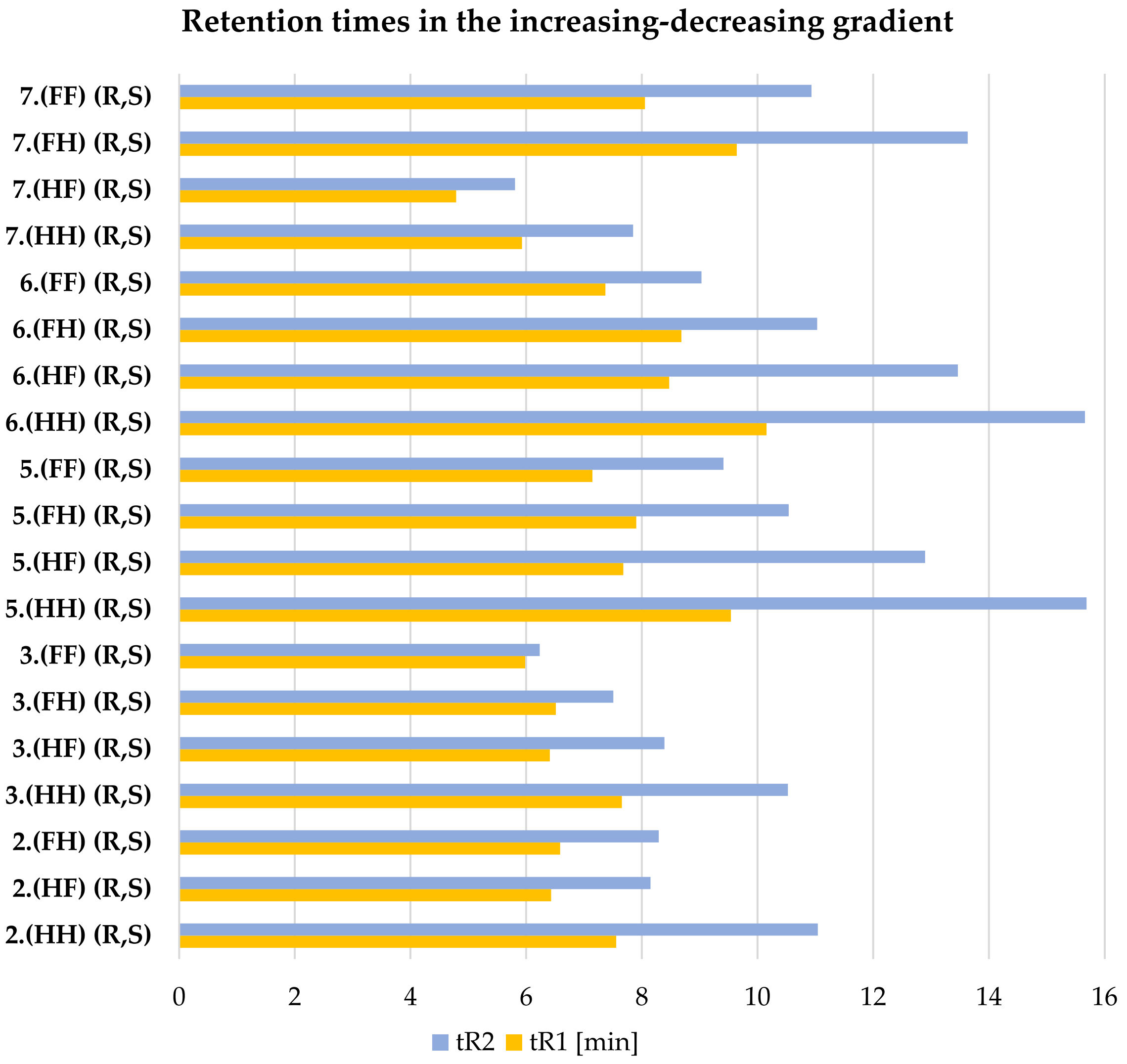


Figure 17. The retention times of the racemic mixtures on the MIG column in the increasing-decreasing gradient.

Figure 18(a-e) shows chromatograms for the mixture 6.(HH) (R,S), in which each system is baseline-separated. In the isocratic elution, as the concentration of acetonitrile in the solvent system decreased, the retention time of the peaks increased. The highest resolution was obtained in the acetonitrile-water system in a volume ratio of 95:5 and the lowest in a volume ratio of 90:10. In the gradient elution, much lower parameters were obtained for the decreasing gradient, which had the lowest values among all systems, and for the increasing-decreasing gradient, the best results among the remaining four were obtained for the ReproSil Chiral-MIG column.

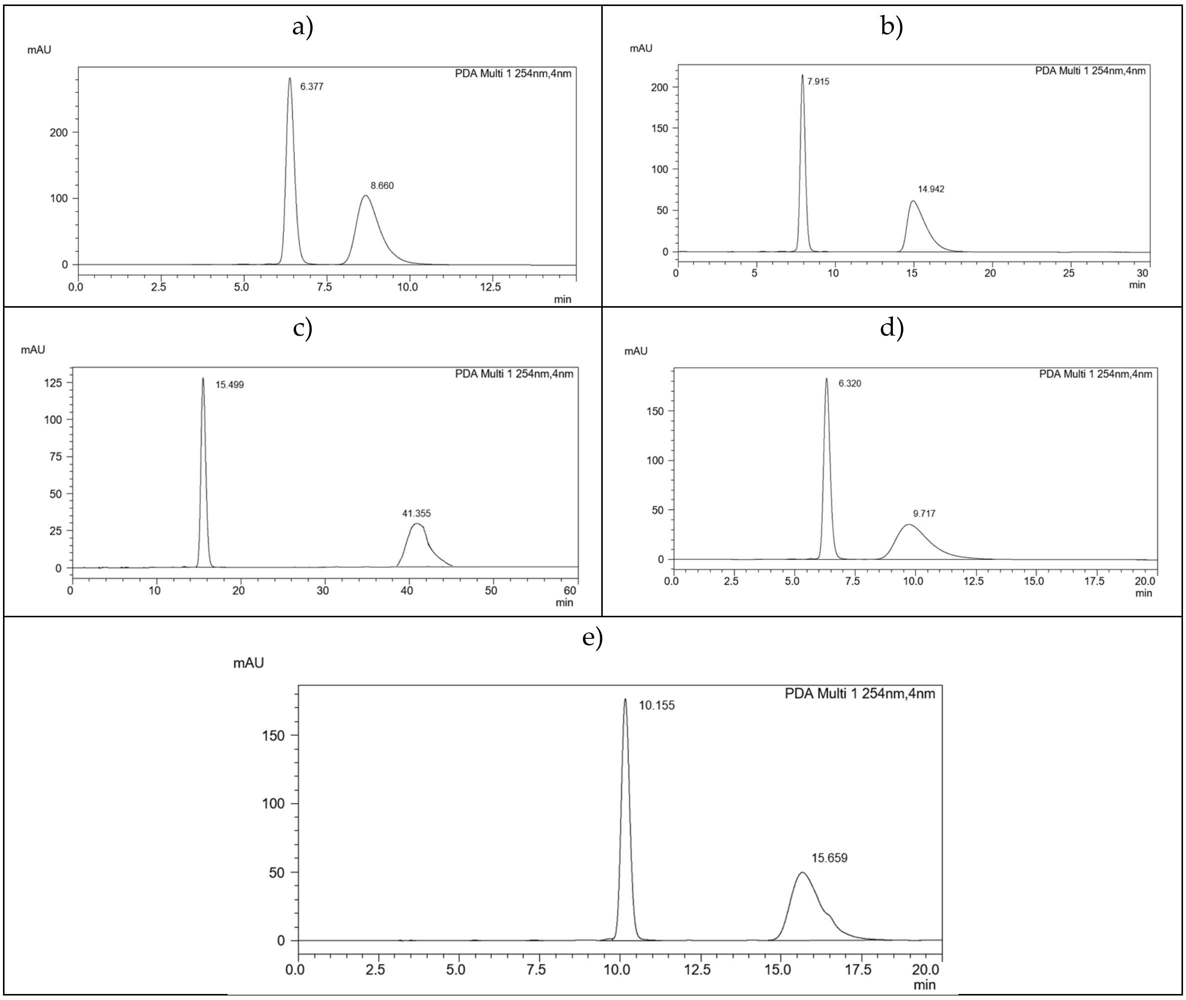


Figure 18. Chromatograms of the racemic mixture 6.(HH) (R,S) obtained on the MIG column in ACN-H_2O solvent systems: a) in a volume ratio of 99:1; b) in a volume ratio of 95:5; c) in a volume ratio of 90:10; d) in the decreasing gradient; e) in the increasing-decreasing gradient.

2.4. ReproSil Chiral-MIC Column

The second chiral column on which the separation of racemic mixtures was continued was the ReproSil Chiral-MIC column. All previously prepared racemic mixtures were separated using the isocratic and the gradient elution. The same analysis parameters were used for the MIC column (as shown in Tables 2 and 3), except that the flow rate for the ACN/H_2O (99:1 v/v) system was reduced to 0.5 mL min^{-1} and for the ACN/H_2O (95:5 v/v) and (90:10 v/v) up to 0.3 mL·min^{-1}.

Analysis of racemic mixtures in non-equimolar ratios allowed the determination of the elution order of the enantiomers. Unlike the ReproSil Chiral-MIG column, the (R) enantiomer was the first to elute during the analysis of the racemic mixtures on the ReproSil Chiral-MIC column (Figure 19).

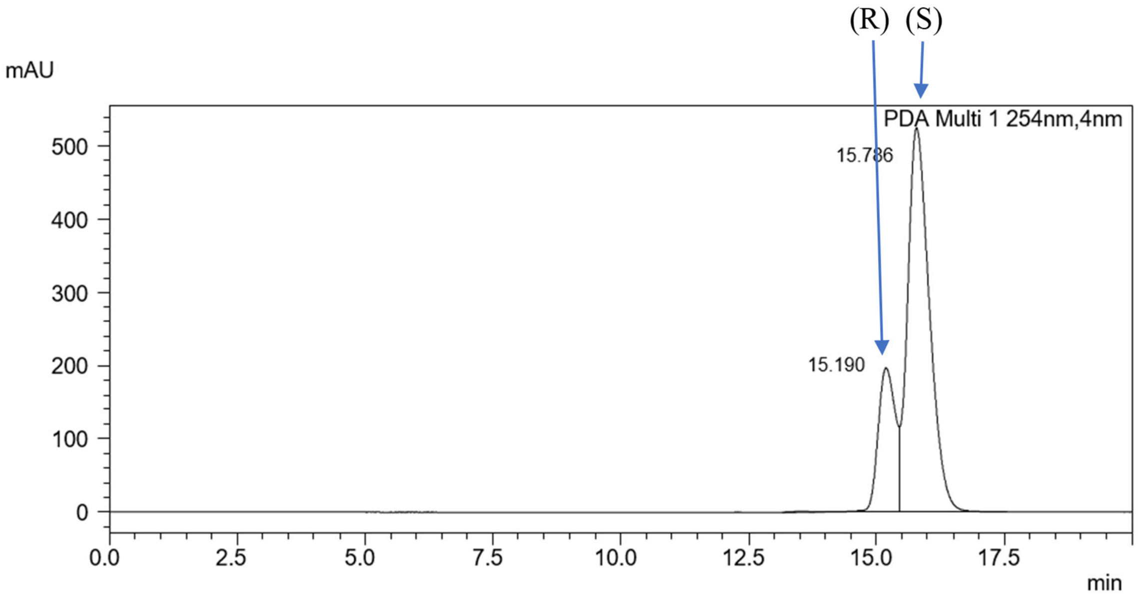


Figure 19. Chromatogram of the mixture 6.(HH) (R,S) + (S) obtained in the isocratic elution, ACN/H_2O (95:5 v/v), the flow rate 0.3 mL·min^{-1}.

On the MIC column, none of the mixtures were baseline-separated regardless of the elution used, and the best results (partial separation of several mixtures) were obtained for the ACN/H_2O (90:10 v/v) system. The comparison of the resolution parameter for both columns and the conditions indicated above is summarized in Table 4 and visualized in Figure 20.

Table 4. The resolution obtained on both columns in the isocratic elution (90:10 v/v).

Resolution		
Acronym of the racemic mixtures	**MIG column**	**MIC column**
2.(HH) (R,S)	4.482	0.611
2.(HF) (R,S)	2.503	0.466
2.(FH) (R,S)	2.561	-
3.(HH) (R,S)	1.428	0.796
3.(HF) (R,S)	3.237	0.582
3.(FH) (R,S)	1.802	0.469
3.(FF) (R,S)	0.900	0.583
5.(HH) (R,S)	1.675	0.836
5.(HF) (R,S)	4.021	0.761
5.(FH) (R,S)	0.928	0.604
5.(FF) (R,S)	3.188	0.628
6.(HH) (R,S)	6.083	0.772
6.(HF) (R,S)	3.425	0.824
6.(FH) (R,S)	3.565	0.640
6.(FF) (R,S)	2.260	0.743
7.(HH) (R,S)	1.689	1.000
7.(HF) (R,S)	1.310	1.073
7.(FH) (R,S)	4.382	-
7.(FF) (R,S)	3.690	0.768

doi:10.20944/preprints202403.0796.v1

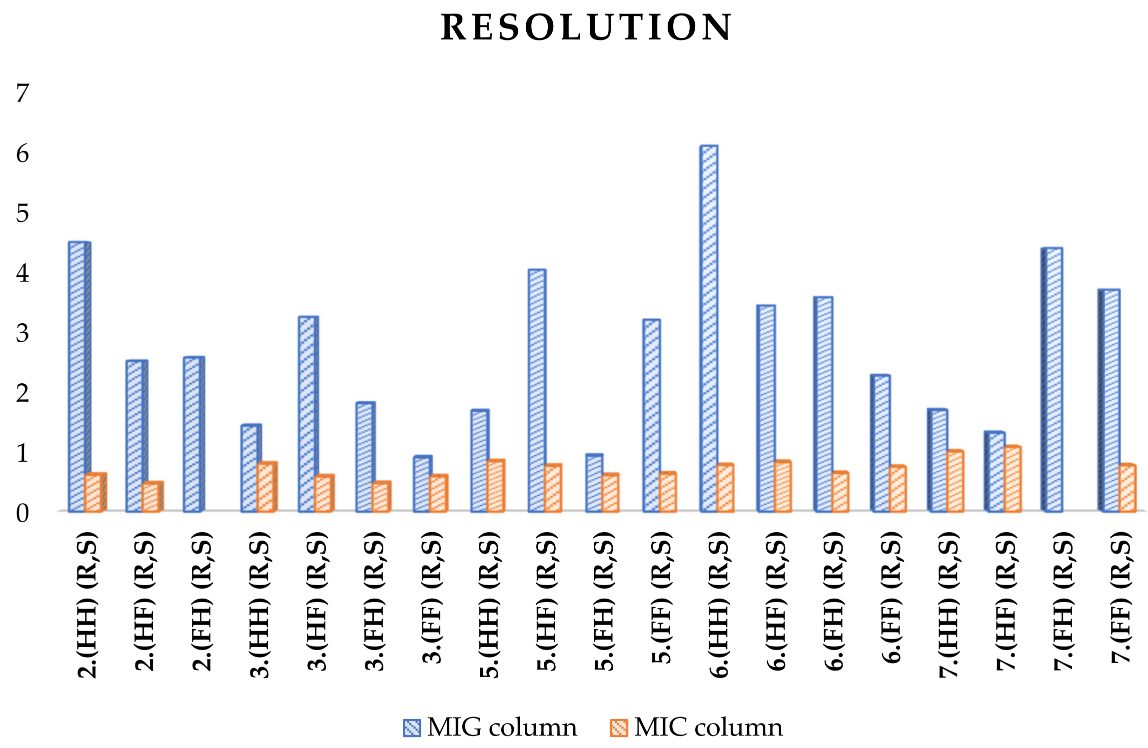


Figure 20. Comparison of the resolution parameter on two columns.

In each case, we have a higher resolution on the amylose column; even reducing the flow rate on the cellulose column did not significantly improve the resolution. The highest resolution (over 6) was obtained for the mixture 6.(HH) (R,S) on the MIG column.

The selectivity parameter for these two columns was also compared, as shown in Figure 21. On the MIC column, this value exceeds at least one; on the MiG column, it is always higher, and in the case of the mixture 6.(HH) (R,S), it exceeds the value of 2.5. Altering the system selectivity provides an excellent means of optimizing the chromatographic resolution, as small changes in selectivity can lead to large changes in resolution.

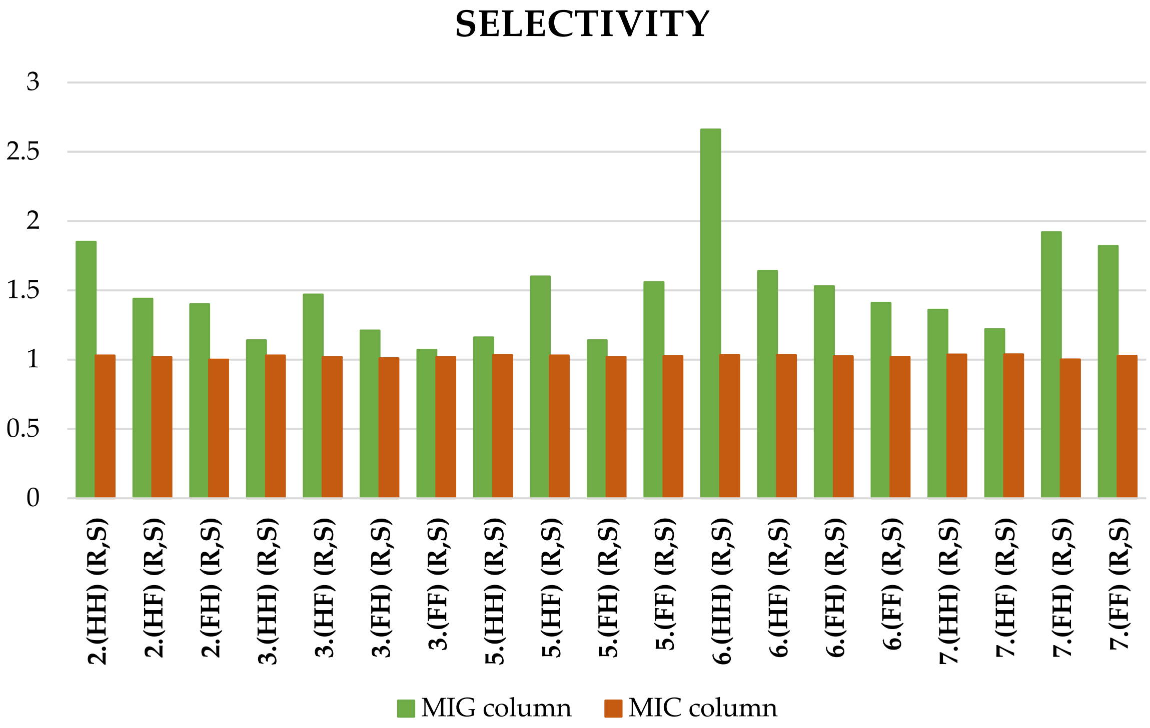
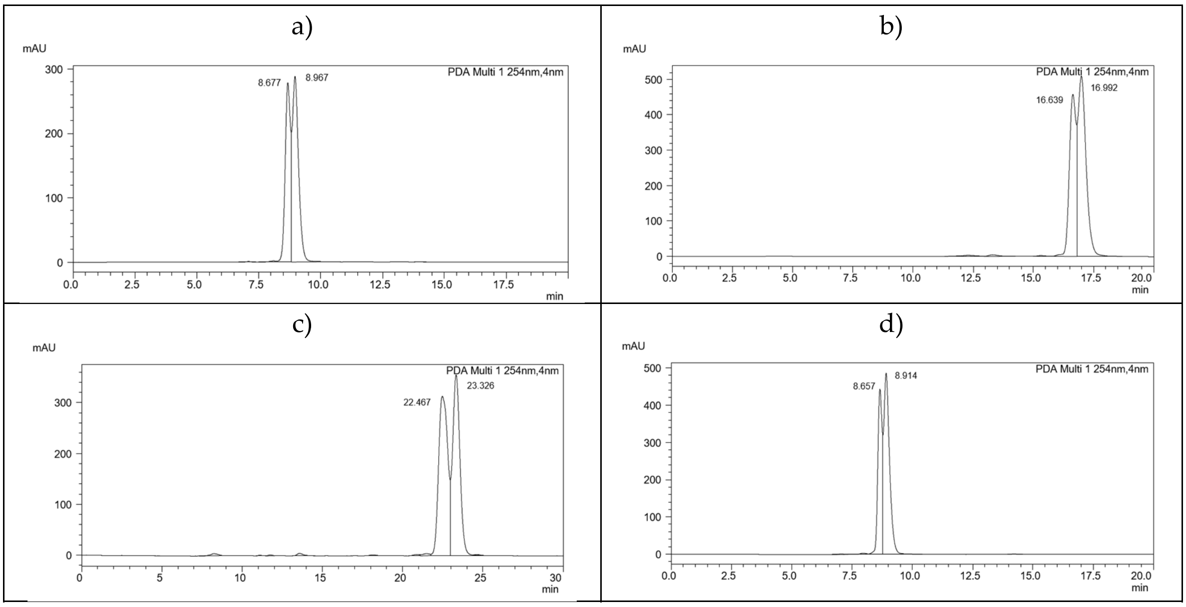


Figure 21. Comparison of the selectivity parameter on two columns.

Figure 22(a-e) shows chromatograms for the mixture 7.(HF) (R,S), which separated best in all five systems considered on the MIC column. In the isocratic elution, as the concentration of acetonitrile in the solvent system decreased, the retention time of the peaks increased. The highest resolution was obtained in the acetonitrile-water system in a volume ratio of 90:10 and the lowest in a volume ratio of 95:5. In gradient elution, much lower parameters were obtained for the increasing-decreasing gradient, which showed similar parameter values to the isocratic system in a volume ratio of 95:5.

In the Supplementary Materials, Figures S18-S42 show the resolution, corresponding chromatograms, retention times, and selectivity for the MIC column in all elution types for all racemic mixtures. The number of theoretical plates was not counted because baseline separation was not observed for any mixture.



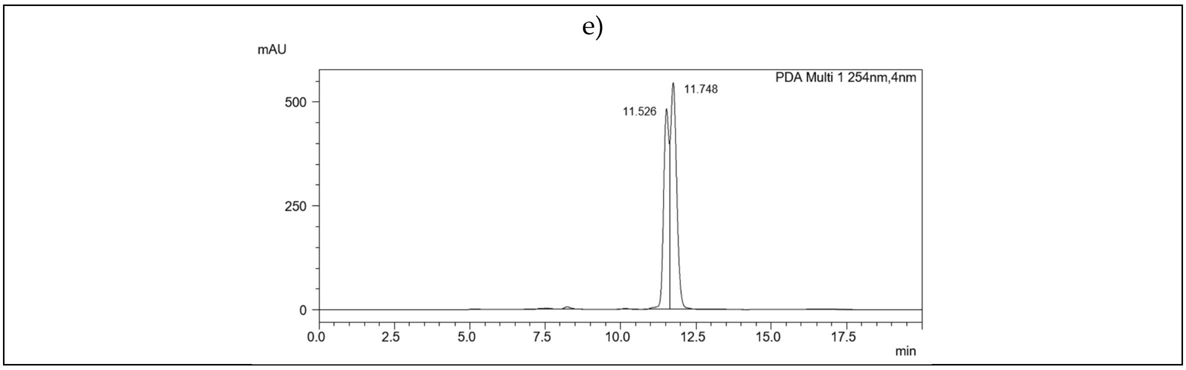


Figure 22. Chromatograms of the racemic mixture 7.(HF) (R,S) obtained on the MIC column in ACN-H_2O solvent systems: a) in a volume ratio of 99:1; b) in a volume ratio of 95:5; c) in a volume ratio of 90:10; d) in the decreasing gradient; e) in the increasing-decreasing gradient.

3. Conclusions

The separation of liquid crystalline racemic mixtures obtained based on (R,S)-2-octanol on both columns is influenced by:

- volume ratio of solvents used,
- chemical structure of the separated racemic mixtures (number of fluorine atoms or their absence in the benzene ring, number of methylene groups),
- the type of elution.

As many as seventeen racemic mixtures were baseline-separated in the increasing-decreasing gradient on the Reprosil Chiral-MIG column. The highest resolution was achieved in this gradient, and such a column could be used for preparative chromatography.

On the ReproSil Chiral-MIC column, none of the racemic mixtures were baseline-separated regardless of the elution used, with the best results obtained for the isocratic elution where the volume ratio of water in the mobile phase was the highest (seventeen mixtures were partially separated).

Regardless of the column used, the longest retention times are observed for non-fluorinated mixtures with type (HH) substitution.

The order of elution of enantiomers depends on the column packing material; in the case of the amylose column, the (S) enantiomer elutes first, while for the cellulose column, the opposite situation occurs.

In summary, the diversity of polysaccharide derivatives has been shown to provide broad chiral recognition for enantiomers, making polysaccharide derivatives the most important CSPs. Moreover, as shown in previous articles [17,42–45], the separation process of liquid crystalline racemates can be carried out using UPLC and SFC techniques, which gives excellent results. In recent years, supercritical liquid chromatography (SFC) has developed particularly well as a separation technique [46–50]. This technique is well known for its fast and ultra-fast, high-throughput separations, improving the environmental friendliness of the analytical method.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org: Figures S1–S42.

Author Contributions: Conceptualization, M.U.; methodology, M.U.; software, M.U.; validation, M.U.; formal analysis, M.U.; investigation, M.U.; resources, M.U.; data curation, M.U.; writing—original draft preparation, M.U.; writing—review and editing, M.U.; visualization, M.U.; supervision, M.U.; project administration, M.U.; funding acquisition, M.U. The author has read and agreed to the published version of the manuscript.

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