Middle East Journal of Science

https://dergipark.org.tr/mejs **MEJS**

e-ISSN:2618-6136

Research Article

PREPARATION AND CHARACTERIZATION OF A NEW SILICA GEL - BASED PIRKLE - TYPE CHIRAL STATIONARY PHASE AS A NEW HPLC COLUMN PACKING MATERIAL

***Ramazan ALTINDAĞ*[1] *Murat SUNKUR*[2] *Reşit ÇAKMAK*[3] *Giray TOPAL**[4]

[1] Department of Chemistry, Institute of Science, Dicle University, Diyarbakır, Türkiye
[2] Department of Chemistry, Faculty of Sciences and Arts, Batman University, Batman, 72060, Türkiye
[3]Medical Laboratory Techniques Prog., Vocational School of Health Services, Batman Uni., Batman, Türkiye
[4]Department of Chemistry, Ziya Gökalp Faculty of Education, Dicle University, Diyarbakır, Türkiye
* Corresponding author: gtopal@dicle.edu.tr

Abstract: *In this study, a new silica-based Pirkle-type chiral stationary phase was successfully prepared by using silica gel 60 as a matrix, 3-(chloropropyl)-trimethoxysilane as a spacer arm, and (S)-4-amino-N-(1-cyclohexylethyl)benzamide (II) a chiral selector; and loaded onto the high performance liquid chromatography column by using a mobile phase to prepare a new chiral high performance liquid chromatography column. The chiral selector was synthesized in two steps. In the first step, (S)-N-(1-cyclohexylethyl)-4-nitrobenzamide(I) was obtained, and then the aromatic amine derivative (II) of this compound was obtained with hydrazine hydrate and Pd/C catalyst. The prepared chiral selector (II) was then immobilized on 3-chloropropyl functionalized silica gel (III) prepared by condensation reaction of 3-(chloropropyl)-trimethoxy silane with silica gel 60. In the last step, the new chiral stationary phase derived from silica (IV) was loaded onto a commercial column with the aid of a column-packing apparatus using known methods. The structures of the synthesized chiral selector (II), the new chiral stationary phase (IV), and the characteristic features of the prepared column were investigated by spectroscopic techniques. As a result of the spectroscopic analysis, it was determined that the new chiral stationary phase was prepared successfully.*

Keywords*: Chirality, Chiral stationary phase, Silica-based chiral high performance liquid chromatography column.*

Received: April 18, 2025 *Accepted: June 4, 2025*

1. Introduction

Chirality refers to a situation in which mirror images of compounds are not superimposable [1]. These compounds usually consist of at least two enantiomers that can have different effects in biological systems [2-4]. Nowadays, chiral high performance liquid chromatography (HPLC) columns containing specially designed chiral stationary phases (CSPs) are used to make enantiomeric resolution of racemic mixtures, which are equal mixtures of two enantiomers [5,6]. In the preparation of chiral HPLC columns, different matrices are used as column packing materials. For example, silica gel is one of them. The surface of silica gel is modified with chiral selectors having chiral groups to create a chiral environment. In this way, the behavior of enantiomers that encounter a chiral environment towards the chiral stationary phase differs. As a result of this differentiation, racemic mixtures can be divided into their enantiomers [7,8].

On the other hand, polymer-based stationary phases are among the important stationary phases used in the preparation of chiral HPLC columns. These stationary phases are usually made of natural

or synthetic polymers and contain suitable chiral groups that can separate enantiomers [9]. Apart from these, other CSPs can be prepared from macrocyclic glycopeptide antibiotics, protein-based structures, ligand exchange-based structures, and polysaccharide-based structures [10]. Chiral ligands are used to enable different interactions between enantiomers by binding to the surface of stationary phases. Generally, these stationary phases are cyclic structures such as crown ethers and cyclodextrins containing chiral cavities. CSPs based on ion exchange are structures in which ion exchange is carried out under the coordination of a dominant metal ion and operate on the basis of ion exchange [11,12]. In recent years, a new stationary phase has been added to these stationary phases. Previously known as Brush-type CSP and later called Pirkle-type stationary phases, these new generation stationary phases are formed by attacking and permanently binding a small chiral organic molecule attached to a support arm such as a 3,5-dinitro benzoyl group to the silica surface, and the chiral groups on the silica surface have a combed brush appearance [13,14]. The enantiomer pair to be separated is physically attached to these surfaces through pi bond interactions, hydrogen bond interactions, ion-dipole interactions. The chiral groups used can be acidic or basic amino acids, as well as chiral amino alcohol, amide alcohol, ester or chiral carboxylic acid derivatives [15].

Pirkle-type CSPs are mostly silica-based materials containing chiral separating selectors [16]. These CSPs were first developed by W. H. Pirkle and colleagues in 1967 and were designed based on the idea that molecules containing chiral groups could be interacted with non-chiral connecting arms or surfaces to form a series of new ligands containing chiral groups [17,18]. These systems enable the separation of enantiomers by using different interactions of optical equivalents. Pirkle ligands are chiral organic molecules usually derived from natural or synthesized compounds [19]. These ligands can provide selective interactions based on the different physicochemical properties of the enantiomers, presenting a specific stereo chemical structure. For instance, some of Pirkle ligands may contain β-amino acid derivatives and other similar chiral structures [20]. In these CSPs, chiral molecules are attached to the silica gel by ionic or covalent bonds. In general, we can talk about three types of Pirkle-type CSPs. These are π-acidic CSPs, π-basic CSPs, and π-acidic-basic CSPs [21]. The first stationary phase of ionic bonding developed by Pirkle is (*R*)-3,5-dinitrobenzoyl phenyl glycine. The most commonly used spacer arm is 3,5-dinitrophenyl group, which is known for its reactions with 3,5-dinitrobenzoyl chloride on chiral selectors including amino acids, amino alcohols, and amines. The first CSP designed based on ionic bonding was designed by Pirkle and contained 3,5-dinitro benzoyl groups a spacer arm [22]. These CSPs contain phenyl or alkyl-substituted phenyl groups. Pirkle-type CSPs are quite stable and have good chiral selectivity. This type of CSP is frequently used for the separation of racemic drugs and amino acid analysis [23]. Firstly, these CSPs achieved chiral separation of racemic compounds. However, in some cases, racemic compounds may need to be previously derivatized with achiral reagents. Experimental studies have shown that this type of CSPs is more effective than other CSPs in chiral separation. In such CSPs, chiral separations are usually performed in normal phase mode. However, with the development of new and more stable phases, the reverse phase mode has become more popular [24-28].

In the current study, a new Pirkle-type CSP was obtained as a new silica gel based column-packing material and the prepared CSP was loaded onto the column to prepare a new chiral HPLC column. In this context, a chiral selector was synthesized and elucidated by spectroscopic techniques including ^{1}NMR, ^{13}C NMR and FTIR. The molecular structure of newly prepared CSP was characterized by FT-IR, elemental analysis and BET measurement, respectively.

2. Materials and Methods

2.1. Chemistry and Analysis

All chemicals (4-nitrobenzoylchloride, (*S*)-(+)-1-cyclohexylethylamine, hydrazine monohydrate (H_2N-NH_2.H_2O), palladium on carbon (Pd/C), tetrahydrofuran, toluene, chloroform, triethylamine, silica gel (Lichrospher Si 60, 5 µm, 60 Å) and 3-chloropropyltrimethoxy silane) used in the preparation of the chiral ligand and the targeted CSP were commercially obtained from Sigma, Merck or Fluka. These chemicals were used without any further purification. Deionized water (Millipore Milli-Q water system) was used in the preparation of all aqueous solutions. Melting points of the targeted compounds were determined by using a Barnstead IA9100 electrothermal digital melting points apparatus. The structures of the synthesized compounds were determined by FT-IR (PerkinElmer Spectrum 100 FT-IR spectrophotometer), ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra (Bruker DPX-400 High Performance Digital FT-NMR spectrometer), and elemental analysis (Thermo Scientific FLASH 2000 instrument). Empty HPLC column was obtained from Quality System Merieux Nutrisciences Company (Istanbul, Turkey). The synthesized chiral stationary phase was packed into the empty HPLC columns using an easypack analytical column packing system (Compressor model DA7001). Specific surface area analysis was made using single-point BET measurements on a Micromeritics Tristar II Plus BET analyzer.

2.2. Synthesis of (*S*)-*N*-(1-cyclohexylethyl)-4-nitrobenzamide (I)

A solution of *p*-nitrobenzoyl chloride (0.0314 mmol) and triethylamine (4.5 mL) in 100 mL of tetrahydrofuran was vigorously stirred at room temperature for 30 min. Then, (*S*)-(+)-1-cyclohexylethylamine (0.0314 mol) in 100 mL of tetrahydrofuran was added dropwise to this solution, and then the obtained reaction mixture was stirred at room temperature for 24 h. After completion of the reaction, the precipitated triethylammonium chloride was removed by vacuum filtration. The resulting filtration was concentrated by rotary evaporation to remove the solvent. The crude product was recrystallized from a 3:2 ethanol/methanol mixture. The product, obtained as off-white needle-like crystals, was collected by vacuum filtration and washed with diethyl ether or petroleum ether (40–60 °C). The final product was dried in air at room temperature.

2.2.1 (*S*)-*N*-(1-cyclohexylethyl)-4-nitrobenzamide

Yield: 6.93 g (79%), melting point: 199–200 °C. FT-IR (cm^{-1}): 3290, 3079 (Aromatic C–H stretching), 2961, 2849 (Aliphatic C–H stretching), 1636 (Amide C=O stretching), 1598 (NO_2 asymmetric stretching), 1546–1442 (C–C stretching, aromatic benzene ring), 1342 (C–H bending vibrations), 1106–1188 (C–N and aromatic C–O stretching). ^{1}H NMR (δ, ppm, $CDCl_3$): 1.23–1.82 (m, 14H, aliphatic H), 4.05–4.10 (m, 1H, CH), 6.14 (d, 1H, NH), 7.92 (d, 2H, Ar–H, J = 2.0 Hz), 8.27 (d, 2H, Ar–H, J = 2.0 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.92, 26.13, 29.18, 43.15, 50.49, 123.76, 128.06, 140.76, 149.43, 164.86.

2.3. Synthesis of (*S*)-4-amino-*N*-(1-cyclohexylethyl)benzamide (II)

To a 500 mL two-necked, round-bottom flask equipped with a reflux condenser, 5.56 g of (*S*)-*N*-(1-cyclohexylethyl)-4-nitrobenzamide was added along with 200 mL of absolute ethanol. The mixture was stirred using a magnetic stirrer under gentle heating until complete dissolution was achieved. Then, 0.5 g of palladium on carbon (Pd/C) was added to this solution, and the reaction temperature was increased to 65 °C. After stirring for 30 min, 100 mL of hydrazine hydrate, previously placed in a 250 mL dropping funnel, was added dropwise over 30 min. The reaction mixture was then refluxed under continuous stirring for 7 h. After completion of the reaction, the

mixture was filtered many times by filtration to remove the Pd/C catalyst until a clear and light-colored filtrate was obtained. The filtrate was transferred to a rotary evaporator, and the solvent was removed under reduced pressure. The solid residue was crystallized from an ethanol/hexane mixture. The resulting product is formed as shiny white, fluffy crystals. The crystals were collected by vacuum filtration and dried in a desiccator.

2.3.1 (*S*)-4-amino-*N*-(1-cyclohexylethyl)benzamide (II)

Yield: 4.56 g (92%). melting point: 180–182 °C. FT-IR (cm^{-1}): 3316 (N–H stretching, doublet), 3200 (Aromatic C–H stretching), 2919, 2849 (Aliphatic C–H stretching), 1624 (Amide C=O), 1538–1446 (C–C stretching, aromatic ring), 1344 (C–H bending), 1283–1273 (Aromatic substitutions). ^{1}H NMR (δ, ppm, $CDCl_3$): 1.10–1.84 (m, 16H, aliphatic), 4.08–4.13 (m, 1H, CH), 5.97 (d, 1H, NH), 7.93 (d, 2H, Ar–H, J = 2.1 Hz), 8.30 (d, 2H, Ar–H, J = 2.1 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.01, 26.23, 29.17, 43.21, 50.42, 123.82, 128.05, 140.75, 149.49, 164.81.

2.4. Preparation of 3-Chloropropyl Functionalized Silica Gel (III)

4 g of silica gel and 2.5 g of 3-chloropropyltrimethoxysilane were added to a 100 mL single neck round bottom flask. Then, 30 mL of toluene was added to this mixture and the reaction mixture was stirred under reflux for 4 days. After the reaction was completed, the mixture was filtered through standard filter paper. The solid was wrapped in double filter paper and placed in the Soxhlet apparatus. The solid was extracted with 150 mL of chloroform overnight. After the extraction process, the product was dried in a desiccator. At the end of the reaction, 5.5 g of 3-chloropropyl functionalized silica gel was obtained.

2.5. Preparation of the Novel CSP (IV)

5.5 g of 3-chloropropyl functionalized silica gel (III) and 2.9 g of compound II dissolved in 40 mL of toluene were added to a 100 mL single neck round bottom flask. The reaction mixture was then stirred under reflux in an oil bath at 130 °C for 6 days. Next, the reaction mixture cooled to room temperature and filtered. The filtrate was washed with methanol to remove unreacted amine. The solid product remaining on the filter paper was then placed in a Soxhlet apparatus and washed with chloroform for 3 days. The resulting yellowish material (CSP) was dried in a desiccator for 7 days. At the end of the reaction, a total of 7 g of new CSP was obtained.

2.6. Preparation of Chiral HPLC Column

Firstly, 3.5 g of the freshly prepared CSP was suspended in 30 mL of methanol. Then, the suspension was loaded onto the column with the help of a column packing apparatus using a 300 mL of methanol: isopropanol (1:1) mixture as the mobile phase. The column filling process was carried out entirely in the column-packing apparatus and at room temperature, and the maximum filling pressure was 500 bar. The flow rate was adjusted by filling the column with the 300 mL suspension for 2 h. Initially, a greenish liquid came from the column during loading. Over time, the color lightened and became clear. Several properties of the newly prepared column are given in Table 1.

Table 1. Properties of the newly prepared chiral HPLC column

Limit Pressure	**500 bar**
[a]Max. flow rate for 100% methanol	2.5 mL/min (250 bar)
[a]Max. flow rate for methanol/isopropanol:50/50	4.5 mL/min (450 bar)
Packing Time	2 h
[b]Particle Size	5 µm
[b]Pore Size	60 Å
Matrix active group	amide, cycloalkyl, aromatic amine
Column dimensions	250 x 4.6 mm
[c]Total pore volume	0.20953 cm^3/g
[c]Surface area	238.1289 m^2/g
[c]Micropore volume	0.04782 cm^3/g
[c]Mesopore volume	0.16171 cm^3/g
[c]Average pore diameter	nm
[d]Extent of labelling	3.73% carbon loading

[a]Max. flow rate was determined at the maximum operation pressure (500 bar) at 25°C.

[b]Physical properties of commercially available silica (unmodified, blank silica) (Sigma-Aldrich).

[c]Based on the BET analysis.

[d]Based on the elemental analysis.

On the other hand, the elemental analysis results of blank silica and silica gel-based new CSP are given in Table 2.

Table 2. The elemental analysis results of blank silica and new CSP

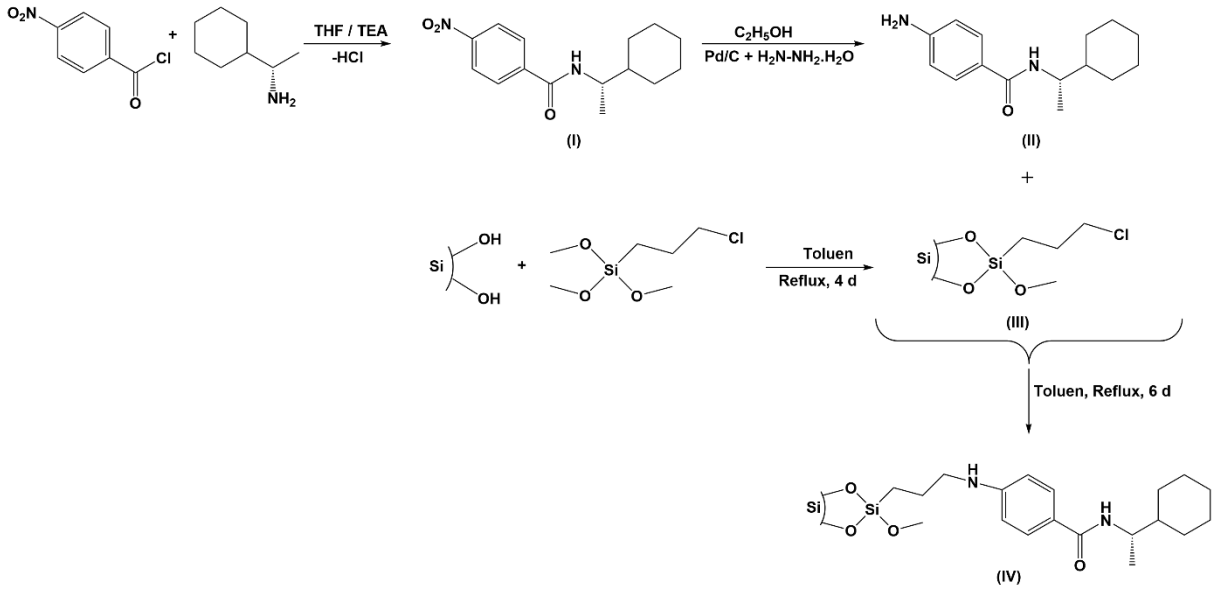
Elemental analysis results (%)	**C**	**H**	**N**
Blank Silica	0	0.04	0
Average	3.73	0.56	0.47

3. Results and Discussion

3.1. Synthesis and Characterization

HPLC separation is among the popular research areas. In recent years, significant efforts have been made to prepare new and efficient HPLC columns. For this purpose, various HPLC columns have been prepared and tested so far using different novel CSPs for various purposes, including enantiomeric resolution or molecular separation [1,7,17,27,29]. In this research, a new Pirkle-type CSP (IV), a new silica gel-based column packing material, was prepared by the condensation reaction of 3-chloropropylfunctionalized silica gel (III) with the chiral selector, and then the prepared novel CSP was loaded onto the HPLC column to obtain a novel chiral HPLC column. To prepare the new CSP, silica gel 60 as a matrix, 3-(chloropropyl)-trimethoxysilane as a spacer arm, and (*S*)-4-amino-*N*-(1-cyclohexylethyl)benzamide (II) as a chiral selector were used. The chiral selector was successfully synthesized in two steps using (*S*)-(+)-1-cyclohexylethylamine as the starting material. The targeted chiral selector and novel CSP were successfully characterized by various spectroscopic techniques.

The synthesis route to obtain the chiral selector (II) and the newly synthesized CSP (IV) is described in Scheme 1.



Scheme 1. Synthetic route to the preparation of the chiral selector (II) and the novel SP (IV)

When examining the IR spectra of compounds used in the synthesis of the chiral selector, while no peaks were observed in the IR spectrum of compound I in the 3300-3500 cm^{-1} region, the characteristic N-H stretching vibrations of primary amines were observed at 3316 cm^{-1} and 3220 cm^{-1} in the IR spectrum of compound II. The 1598 cm^{-1} stretching vibration peak belonging to the nitro group seen in the IR spectrum of compound I disappeared in compound II. Moreover, the observation of amine N-H in-plane bending vibrations at 1602 cm^{-1} in the IR spectrum of compound II indicates that reduction has occurred. In the IR spectrum of compound III, only bands belonging to C-H stretching vibrations (aliphatic C-H stretching is more dominant) are visible around 2971 $cm^{-1,}$ while new peaks were observed at 1610 cm^{-1}, 1506 cm^{-1} and 1448 cm^{-1} in the IR spectrum of compound IV. The new peaks were evaluated as C=O stretching of amide carbonyl, C-H bending of alkyl groups and aromatic ring C=C stretching vibrations, respectively. No change was observed in the basic peaks in the fingerprint region. This is evidence that the amine group was replaced by the chlorine atom in the chlorinated silica (III) as a result of a nucleophilic reaction and that compound II was bound to the silica surface. The fact that the comparative IR spectra of compound III and compound IV do not overlap is another proof that binding has occurred. In the NMR spectra of the chiral selector, the increase in the number of H atoms in the ^{1}H NMR spectrum of compound II obtained as a result of the reduction reaction is another evidence that the NO_2 group has transformed into the NH_2 group. In ^{13}C NMR spectra of the chiral selector (II), the signal at 164 ppm belongs to the carbonyl group, and the signals between 18 and 50 ppm belong to aliphatic carbons. In addition, the peak at 5.97 ppm in the ^{1}H NMR spectrum belongs to the amide proton in the structure. On the other hand, the specific surface area and the total pore volume of the new CSP measured by using BET analysis were calculated to be 238.1289 m^2g^{-1} and 0.20953 $cm^3\ g^{-1}$, respectively, which are greatly reduced compared to those of the blank silica (737 m^2g^{-1} and 0.75 cm^3g^{-1})[7], as seen in Table 1. In addition, elemental analysis of blank silica and new CSP was carried out to measure the elemental composition. As shown in Table 2, the carbon (C), hydrogen (H) and nitrogen (N) contents of the new CSP were calculated as 3.73 0.56 0.47, respectively. FTIR, BET measurement and the elemental analysis results of novel silica gel-based CSP

demonstrate the successful preparation of the targeted column packing material. FT-IR, ^{1}H, and ^{13}CNMR spectra of chiral selector (I and II), 3-chloropropyl functionalized silica gel (III) and novel CSP (IV) are given in the Supplementary Materials, Figures S1−S9.

4. Conclusion

In this study, a new Pirkle-type CSP (IV) as a new silica gel-based column packing material was easily obtained by the reaction of a chiral selector (II) with 3-chloropropyl functionalized silica gel (III). Then, the prepared new CSP (IV) was loaded onto the HPLC column using a mobile phase. For this study, the chiral selector was synthesized in two steps and in high purity, and then elucidated by spectroscopic techniques (^{1}NMR, ^{13}C NMR and FTIR). The structure of the targeted novel CSP was characterized by FT-IR, elemental analysis and BET measurement. At the end of the study, we can say that the newly prepared chiral HPLC column has the potential to be used in enantiomeric resolution of racemic compounds or molecular separation studies of bioactive or important molecules.

Ethical statement

The author declares that this document does not require ethics committee approval or any special permission. This study does not cause any harm to the environment.

Acknowledgment

The authors would like to thank Dicle University Scientific Research Projects Coordination Unit for the financial support provided to them through project number **FBE.21.008**.

Conflict of interest:

The article's authors declare that there is no conflict of interest between them.

Authors' Contributions:

R. A: Methodology, Validation, Formal analysis, Investigation (%35)

M. S: Validation, Formal analysis, Methodology, Investigation, Writing - original draft preparation (%25)

R. Ç: Validation, Formal analysis, Writing - original draft preparation (%10)

G. T: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition (%30)

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

References

[1] Yılmaz, H., Topal, G., Çakmak, R., Hosgoren, H., Resolution of (±)-β-methylphenylethylamine by a novel chiral stationary phase for Pirkle-type column chromatography. *Chirality*: The Pharmacological, Biological, and Chemical Consequences of Molecular Asymmetry, 22(2), 252-257, 2010.

[2] Basaran, E., Sogukomerogullari, H.G., Çakmak, R., Akkoc, S., Taskin-Tok, T., Köse, A., "Novel chiral Schiff base Palladium (II), Nickel (II), Copper (II) and Iron (II) complexes: Synthesis, characterization, anticancer activity and molecular docking studies", *Bioorganic Chemistry*, 129, 106176, 2022. https://doi.org/10.1016/j.bioorg.2022.106176.

[3] Başaran, E., Karaküçük-Iyidoğan, A., Schols, D., Oruç-Emre, E.E., "Synthesis of novel chiral sulfonamide-bearing 1, 2, 4-triazole-3-thione analogs derived from D-and L-phenylalanine esters as potential anti-influenza agents", *Chirality*, 28(6), 495-513, 2016. https://doi.org/10.1002/chir.22607.

[4] Karaküçük-İyidoğan, A., Başaran, E., Tatar-Yılmaz, G., Oruç-Emre, E.E. "Development of new chiral 1, 2, 4-triazole-3-thiones and 1, 3, 4-thiadiazoles with promising in vivo anticonvulsant activity targeting GABAergic system and voltage-gated sodium channels (VGSCs)", *Bioorganic Chemistry*, 151, 107662. 2024, https://doi.org/10.1016/j.bioorg.2024.107662.

[5] Okamoto, Y., Ikai, T., "Chiral HPLC for efficient resolution of enantiomers", *Chemical Society Reviews*, 37(12), 2593-2608, 2008. https://doi.org/10.1039/B808881K.

[6] Lomenova, A., Hroboňová, K., "Comparison of HPLC separation of phenylalanine enantiomers on different types of chiral stationary phases", *Food Analytical Methods*, 11, 3314-3323, 2018. https://doi.org/10.1007/s12161-018-1308-9.

[7] Aral, H., Çelik, K.S., Altındağ, R., Aral, T., "Synthesis, characterization, and application of a novel multifunctional stationary phase for hydrophilic interaction/reversed phase mixed-mode chromatography", *Talanta*, 174, 703-714, 2017. https://doi.org/10.1016/j.talanta.2017.07.014.

[8] Lorenz, H., Seidel-Morgenstern, A., "Processes to separate enantiomers", *Angewandte Chemie International Edition*, 53(5), 1218-1250, 2014, https://doi.org/10.1002/anie.201302823.

[9] Wang, Y., Chen, H., Xiao, Y., Ng, C.-H., Oh, T.S., Tan, T.T.Y., Ng, S.C., "Preparation of cyclodextrin chiral stationary phases by organic soluble catalytic'click'chemistry", *Nature protocols*, 6(7), 935-942, 2011. https://doi.org/10.1038/nprot.2011.340.

[10] Ali, I., Kumerer, K., Aboul-Enein, H.Y., "Mechanistic principles in chiral separations using liquid chromatography and capillary electrophoresis", *Chromatographi*a, 63, 295-307, 2006. https://doi.org/10.1365/s10337-006-0762-5.

[11] Ward, T.J., "Chiral separations", *Analytical Chemistry*, 72(18), 4521-4528, 2000. https://doi.org/10.1021/ac000841o.

[12] Kuhn, R., Stoecklin, F., Erni, F., "Chiral separations by host-guest complexation with cyclodextrin and crown ether in capillary zone electrophoresis", *Chromatographia*, 33, 32-36, 1992. https://doi.org/10.1007/BF02276847.

[13] Wolf, C., Pirkle, W.H., "Synthesis and evaluation of a copolymeric chiral stationary phase", *Journal of Chromatography A*, 799(1-2), 177-184, 1998. https://doi.org/10.1016/S0021-9673(97)01063-7.

[14] Pirkle, W.H., Burke Iii, J.A., "Chiral stationary phase designed for β-blockers", *Journal of Chromatography A*, 557, 173-185, 1991. https://doi.org/10.1016/S0021-9673(01)87131-4.

[15] Pirkle, W.H., Sikkenga, D.L., "Resolution of optical isomers by liquid chromatography", *Journal of Chromatography A*, 123(2), 400-404, 1976. https://doi.org/10.1016/S0021-9673(00)82210-4.

[16] Vedovello, P., Costa, J.A.S., Fernandes, C., Tiritan, M. E., Paranhos, C.M., "Evaluation of chiral separation by Pirkle-type chiral selector based mixed matrix membranes", *Separation and Purification Technology*, 289, 120722, 2022. https://doi.org/10.1016/j.seppur.2022.120722.

[17] Çakmak, R., Ercan, S., Sunkur, M., Yilmaz, H., Topal, G., "Design, preparation and application of a Pirkle-type chiral stationary phase for enantioseparation of some racemic organic acids and

molecular dynamics studies", *Organic Communications*, 10(3), 216–227, 2017. http://doi.org/10.25135/acg.oc.25.17.07.0.

[18] Pirkle, W.H., Pochapsky, T.C., "Considerations of chiral recognition relevant to the liquid chromatography separation of enantiomers", *Chemical Reviews*, 89(2), 347-362, 1989. https://doi.org/10.1021/cr00092a006.

[19] Zhao, Y., Zhu, X., Jiang, W., Liu, H., Wang, J., Sun, B., "Natural and artificial chiral-based systems for separation applications", *Critical Reviews in Analytical Chemistry*, 53(1), 27-45, 2023. https://doi.org/10.1080/10408347.2021.1932408.

[20] Peter, A., Lazar, L., Fülöp, F., Armstrong, D.W., "High-performance liquid chromatographic enantioseparation of β-amino acids," *Journal of Chromatography A*, 926(2), 229-238, 2001. https://doi.org/10.1016/S0021-9673(01)01078-0.

[21] Vargas-Caporali, J., Juaristi, E., "Fundamental Developments of Chiral Phase Chromatography in Connection with Enantioselective Synthesis of β-Amino Acids", *Israel Journal of Chemistry,* 57(9), 896-912, 2017. https://doi.org/10.1002/ijch.201700011.

[22] Simeonov, S.P., Simeonov, A.P., Todorov, A.R., Kurteva, V.B., "Enantioresolution of a series of chiral benzyl alcohols by HPLC on a dinitrobenzoylphenylglycine Stationary Phase after Achiral Pre-Column Derivatization", *American Journal of Analytical Chemistry*, 1(1), 1-13, 2010. https://doi.org/10.4236/ajac.2010.11001.

[23] Mane, S., "Racemic drug resolution: a comprehensive guide", *Analytical Methods*, 8(42), 7567-7586, 2016. https://doi.org/10.1039/C6AY02015A.

[24] Aral, H., Çelik, K.S., Aral, T., Topal, G., "Preparation of a novel ionic hybrid stationary phase by non-covalent functionalization of single-walled carbon nanotubes with amino-derivatized silica gel for fast HPLC separation of aromatic compounds", *Talanta*, 149, 21-29, 2016. https://doi.org/10.1016/j.talanta.2015.11.029.

[25] Ilisz, I., Aranyi, A., Péter, A., "Chiral derivatizations applied for the separation of unusual amino acid enantiomers by liquid chromatography and related techniques", *Journal of Chromatography A*, 1296, 119-139, 2013. https://doi.org/10.1016/j.chroma.2013.03.034.

[26] Colin, H., Guiochon, G., "Introduction to reversed-phase high-performance liquid chromatography*", Journal of Chromatography A*, 141(3), 289-312, 1977. https://doi.org/10.1016/S0021-9673(00)93537-4.

[27] Erdoğan, Ö., Topal, G., Çakmak, R., Sünkür, M., Canpolat, M., "The preparation of a new protein based HPLC column packing material and enantiomeric resolution of some pharmaceutical related compounds via this column", *Middle East Journal of Science*, 2(1), 77-92, 2016. https://doi.org/10.23884/mejs/2016.2.1.07.

[28] Engelhardt, H., Jungheim, M., "Comparison and characterization of reversed phases", *Chromatographia*, 29, 59-68, 1990. https://doi.org/10.1007/BF02261141.

[29] Kalili, K. M., de Villiers, A., "Recent developments in the HPLC separation of phenolic compounds", *Journal of Separation Science*, 34(8), 854-876, 2011. https://doi.org/10.1002/jssc.201000811.