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HPLC separation of enantiomers using chiral stationary phases

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SUMMARY

HPLC separation of enantiomers using chiral stationary phases
During recent few years, separations of enantiomers have become one of the most important analytical tasks. Modern separation techniques, such as the chromatographic ones, represent a very powerful tool in this respect and nowadays they are commercially and widely used all over the world. The importance of enantioseparation must be emphasized, not just because of drugs, food additives, and pesticides, but also because of other compounds which affect our life. The use of direct chromatographic enantioseparation seems to be a suitable approach not just in the analytical but also the preparative scale. This paper is focused on the separation of enantiomers using the chiral stationary phase as one of the ways of how to perform direct chromatographic separation.
Key words: HPLC – chiral separation – enantiomers – chiral stationary phases – optically active compounds

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SOUHRN

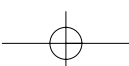**HPLC separácia enantiomérov použitím chirálnych stacionárnych fáz**
Za obdobie posledných rokov sa problematika separácie enantiomérov stala jednou z najdôležitejšie riešených analytických úloh. Moderné separačné techniky, akými sú chromatografické, predstavujú veľmi účinný nastroj a pri separácií enantiomérov sa stali komerčnými a do značnej miery využívanými po celom svete. Význam enantioseparácie je treba zdôrazniť obzvlášť v prípadoch ak sa jedná o analýzu liečiv, potravinárskych aditív, ale aj pesticídov a iných látok s priamym dopadom na naše zdravie. Použitie priamej chromatografickej enantioseparácie sa zdá byť vhodným prístupom nielen v prípade analýzy takýchto látok ale aj v prípade ich preparatívneho delenia. V tomto príspevku je pozornosť upriamená na využitie rôznych chirálnych stacionárnych fáz pri separácií enantiomérov, ako na jeden zo spôsobov, ako je možné uskutočniť priamu chromatografickú separáciu opticky aktívnych látok.
Kľúčové slová: HPLC – chirálna separácia – enantioméry – chirálna stacionárna fáza – opticky aktívne zlúčeniny

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Molecular chirality is a fundamental phenomenon which plays an important role in biological processes. A wide range of biological and physical functions is generated through precise molecular recognition because enzymes, receptors and other natural binding sites within biological systems interact with different enantiomers in decisively different ways. As a result of such chiral recognition, a drug enantiomer may differ in its pharmacological and/or toxicological profiles [1]. In many cases, only one isomer in a chiral compound is responsible for the desired activity, while the other isomer may exhibit no therapeutic value and may potentially cause unsuspected adverse effects [2, 3]. Because of the different biological activities of enantiomers, the preparation of highly enantio-pure compounds is of utmost importance [4].

However, nowadays this issue influences not only the pharmaceutical industry, but also the agrochemical, petrochemical, beverage, food industries, especially food additive producers, all of which are increasingly concerned by this subject.

The increasing demand for the separation of chiral compounds and the production of enantiomerically pure compounds in the fields of pharmacology, chemistry, biotechnology, chemical engineering, etc. has led to enantioselective separation becoming one of the most important analytical tasks [5]. In recent years, various chromatographic and electrophoretic methods have been employed for the separation of enantiomers [6, 7].

Of the many known methods of chiral separation, high-performance liquid chromatography (HPLC) with chiral stationary phases (CSP) is a suitable approach for enantiomeric separation in the analytical or preparative scale for a number of different chiral compounds [8].

Chirality and biological activity

Molecules that relate to each other as an object and its nonsuperimposable mirror image are enantiomers or chirals (from the Greek word, *cheiro,* meaning hand); they are like a pair of hands [9]. There are several proofs that indicate the presence of chirality in our environment. Even in the ancient kingdoms of Upper and Lower Egypt, there are many examples of burial chamber mural paintings that depict significant events in our modern view of chirality [10]. Additionally, out of the 1168 galaxies listed in the *Carnegie Atlas of Galaxies*, 540 are chiral when coupled with the direction of their recession velocities [11]. Numerous examples of asymmetric structures can be observed even among the animals and plants. For example, the helical structures of plants and animals make them asymmetric in nature. However, chirality exists almost everywhere in the universe and there is numerous evidence explaining the origin of the homochirality of biological molecules [12].

Chiral molecules are of different nature and shape, but they can all be classified, on the basis of symmetry elements, into three categories, i.e. exhibiting central, axial, or planar chirality. Three-dimensional space can be occupied asymmetrically about a chiral centre, a chiral axis or a chiral plane. In addition, steric crowding in a molecule may lead to molecular distortion and hence chirality, as found, for example, in helicenes [13, 14].

Generally, it is well known that enantiomers have identical physical properties except for a plus or minus sign of the optical rotation. This optical activity results from the refraction of right and left circulatory polarized light to different extents by chiral molecules. Optical resolution of racemates (a racemate is a mixture consisting of equal amounts of enantiomers) is one way to obtain pure enantiomers.

Since the year 1848, when Luis Pasteur first reported the separation of racemic sodium ammonium tartrate as an example of optical resolution, a great number of compounds have been resolved, mainly by fractional crystallization of diastereomeric salts [15]. From Pasteur's very first optical resolution of a racemate to today's fast chromatographic techniques there has been a formidable accumulation of stereochemical knowledge. In general, chromatographic methods are considered to be the most useful techniques suitable for enantiomeric resolution.

It is not uncommon for one isomer of a racemic compound to produce the desired effect, while another may be inactive or even produce undesired effects. Old examples of a different physiological behaviour are found in the taste and smell of optical antipodes. For example, it is well known that sodium L- (+) glutamate tastes good, whereas its mirror image, D- (-) glutamate, tastes bitter or flat [9].

Enantioseparation using CSPs

The classical methods have not been able to achieve the status of routine laboratory practice due to certain drawbacks. The most important drawbacks associated with these methods are namely the degradation of one enantiomer in the enzymatic method and limitation of applications of the crystallization method. Nowadays, chromatographic, electrophoretic, spectroscopic, biosensing, and membrane methods are the most common techniques applied in this area [16, 17–21].

Chromatographic methods are considered most useful for an enantiomeric resolution for a number of reasons, the most important being the ease of separation. It is generally possible to find a chromatographic method that can provide separation in a matter of minutes. All advantages that chromatographic methods definitely have are due to the flexibility and efficiency that these methods offer. Moreover, these methods can easily be used to determine the enantiomeric ratio of chiral compounds in different matrices.

The theory of chiral chromatography is still rather fundamental, because it is not always entirely clear why, e.g., during the separation a chiral stationary phase selectively retains more of one enantiomer than another. A number of chiral recognition models have been proposed and some explanations have already been given in previous chromatographic studies. Most of them are based on the "three-point interactions" theory advanced by Dalgiesh in 1952 [14]. The three-point interactions are frequently referred to as the three-points rule. This approach considers chiral recognition as it might occur between small chiral molecules in solution [22]. The three-point rule presents

chiral recognition with at least three simultaneous interactions, at least one of which is stereochemically dependent, between a chiral selector and one of the enantiomers whose configuration is to be "recognized"[23].

The most important classes of chiral selectors widely used in chromatography include polysaccharides, cyclodextrins, proteins, Pirkle-type CSPs, ligand exchangers, crown ethers, macrocyclic antibiotics, and several other types, which are not discussed in this paper.

The basic requirements for a suitable chiral selector are as follows: it should be easily available, inexpensive; it should have sufficient groups, atoms, grooves, cavities and so on.

Polysaccharides

The polysaccharides are chiral and optically active because of their asymmetric structures and they have already found some perspectives in the chiral separation of chiral compounds as a suitable chiral stationary phase [24]. These polymers often possess a specific conformation or a higher order structure arising from chirality that is essential for the chiral analysis of racemic compounds [25]. Polysaccharide polymers, such as cellulose, amylose, chitosan, xylan, curdlan, dextran and inulin, have been used for chiral separation in chromatography [26]. Among the various polymers of polysaccharides, cellulose and amylose are the most readily available naturally occurring forms, and they have been found to be suitable for chiral separations. During past two decades, a lot of derivatives of these polymers have been synthesized [25].

Four types of derivates can be easily prepared by modification of the free hydroxyl groups: carbamates, esters, ethers, and nitrates. Ichida *et al.* [27] suggested the following applications for the cellulose-based phase:

Triacetate: especially effective for a substrate with a phosphorous atom as a stereogenic centre.

Tribenzoate: useful for racemates with a carbonyl group near a stereogenic centre.

Trisphenylcarbamate: suitable for polar racemates and sensitive to molecular geometry.

Tribenzyl: effective with protic solvents as mobile phases.

Tricinnamate: useful for aromatic racemates and barbiturates (yields high retention times).

Cellulose tris (3, 5-dimethylphenylcarbamate): is especially suitable for β-adrenergic blocking agents.

For the complete series of cellulose derivates, the mobile phase may be aqueous or non-aqueous. In general, suitable solvents are limited to hexane, hexane/2-propanol (99 to 50% hexane), and methanol or ethanol or mixtures of these with water.

A nonpolar mobile phase usually provides greater resolution than methanol/water mixtures, but lower resolution than ethanol [13]. Here are given just few examples of using polysaccharides chiral stationary for separation of amines [28], amino acids [29], phosphoramidate diastereomers [30], chiral sulfoxides [8], benzothiadiazepin derivates [31], and other drug enantiomers [32, 33–38].

Cyclodextrins

Cyclodextrins (CDs) are chiral, toroidal-shaped molecules composed of six or more D (+)-glucose residues bonded through α-(1,4) glycosidic linkages in which all of the glucose units are held in a C-1 (D) chair conformation [39]. Schardinger discovered that new crystalline carbohydrates, so-called dextrins, were formed if starch was subjected to degradation by the microorganism *Bacillus macerans* [40]. Moreover, Schardinger identified three different forms of naturally occurring CDs – α-, β- and γ-CDs – and referred to them as Schardinger's sugars. They are also called cyclohexamylose (α-CD), cycloheptamylose (β-CD), cyclooctamylose (γ-CD), cycloglucans, glucopyranose, and Schardinger dextrins. The complex formation of CDs and their binding constants have been determined and are controlled by several different factors–hydrophobic interactions, hydrogen bondings and van der Waals interactions [21]. Therefore, CDs and their derivatives have been widely used in separation science since the early 1980s [41, 42]. The presence of a chiral hollow basket/cavity in these molecules makes them suitable for chiral separation of a wide range of chiral molecules. CDs have been used in the form of chiral stationary phases (CSPs) and chiral mobile phase additives (CMPAs).

In 1984, Armstrong developed a hydrolytically stable linkage, where the spacer arm is attached to the silica gel through a silane linkage. An epoxy silane, an organohalosilane or a vinyl silane may be used [43].

In recent years, derivatizing the CD-bonded silica gel has extended the useful range of the CD phases. Five different functional pendant groups have been placed on the CD, i.e., acetyl, 2-hydroxypropyl, naphthylethyl carbamate, dimethylphenyl carbamate, and para- toluyl ester. These derivatized CD columns have been used in both the reversed and normal phase mode making them the first successful multimodal CSPs [44]. A lot of chiral resolutions on CD-based CSPs have been carried out using aqueous mobile phases [45, 46]. Buffers of different concentrations and pH values have been developed and used for this purpose. Triethylammonium acetate (TEAA), phosphate, citrate and acetate are among the most commonly used buffers [41, 42, 47, 48]. In addition, phosphate buffers, such as sodium, potassium and ammonium phosphate, are commonly used. The stability constant of the complexes decreases due to the addition of organic solvents and hence the organic modifiers are used to optimize the chiral resolution [21]. The most commonly used organic solvents are methanol and acetonitrile. Acetonitrile is a stronger organic modifier than methanol. Some other organic modifiers such as ethanol, 2-propanol, 1-propanol, n-butanol, tetrahydrofuran, triethylamine and dimethylformamide have also been used for the optimization of chiral resolution on CD-based CSPs [41, 42]. The effect of the type and concentration of these organic modifiers varies from one analyte to another, and hence it is very difficult to predict the best strategy for their use as organic modifiers [21].

Proteins

Proteins are natural polymers and are made of amino acids – which are chiral molecules, with the exception of glycine-through amide bonds. The complex structures of proteins, which have different types of groups, loops and bridges, are responsible for the chiral resolution of racemic compounds. It has been known for some time that binding to proteins involves multiple equilibria because proteins have a number of binding sites and some of those sites are likely to have different affinities for the ligand [23].

However, enantioselective interactions between proteins and small molecules in different biological systems are well known. These bindings are also responsible for different types of loops/grooves that are present in the protein molecule. This sort of twisted three-dimensional structure of the protein makes it enantioselective in nature [21].

Since 1958, when the enantioselectivity of the binding of the small ligands to bovine serum albumin (BSA) was observed, and since 1973, when the first affinity-type chromatographic separation of enantiomers was described, proteins CSPs have become useful and nowadays they are commercially available for enantioseparations [49, 50–53].

The albumin proteins used as chiral selectors in chromatography are bovine serum albumin (BSA), human serum albumin (HSA), rat serum albumin (RSA) and guinea pig serum albumin (GPSA), but BSA and HSA have been found to be the successful chiral selectors. However, other protein molecules have been explored for their chiral separation capacities; they are glycoproteins, such as α1-acid glycoprotein (AGP), ovomucoid (OVM), ovotransferin, avidin, and trypsin (CT), and certain enzymes, such as chymotrypsin, riboflavin, lysozyme, pepsin, amyloglucosidase, and lactoglobulin. Additionally, cellobiohydrase-I (CBH-I), a protein obtained from fungi, has also been used as a chiral selector in HPLC [52]. Protein-based CSPs are usually carried out under the reversed phase mode, i.e., aqueous mobile phases with buffers of differing concentrations and pH values are frequently used [54, 55].

Brush types-Pirkle type

In 1960, Klemm et al. as the first published the applications of the charge transfer type of complexation to optical resolution by HPLC[57, 58]. In 1976, Mikeš et al. [59] introduced a new concept by attaching a small chiral molecule to silica gel. In this CSP, the organic groups of the chiral molecule remain directed away from the silica gel, appearing in the form of a brush, and hence this is called a brush type phase [21]. Later, Pirkle and co-workers developed these types of CSP extensively, and nowadays they are known as Pirkle-type CSPs [60, 61–65]. Pirkle made a significant breakthrough in charge transfer based adsorption for optical resolution in HPLC by using N-(3,5-dinitrobenzoyl) amino acids as CSPs for π-π interactions based on their work with anthryl carbinol [66, 67]. If one chiral molecule is to recognize the stereochemistry of a second one at some instant in time, three or more simultaneous intermolecular interactions are necessary, at least one of these being stereochemically dependent. If the three interaction sites are construed as points and lie in a plane (achiral plane), that portion of the molecule which lies outside of this hypothetical plane confers chirality to the molecule and impedes back side approach by the second molecule [68, 69]. As mentioned earlier, enantioselective sorption stems from the formation of transient diastereomeric complexes (between the enantiomer and the CSP), which differ in stability.

The enantiomer which is more strongly sorbed is frequently the one that interacts with a greater number of bonding sites or a fewer number of steric repulsion sites [70]. Major interactions sites are classifiable as π-basic or π-acidic aromatic rings, acidic sites, basic sites, steric interaction sites, or sites for electrostatic interactions. Lipophilic interactions are also possible in the reversed mobile phase. In the case of brush-type CSPs, the chiral molecule attached to the silica gel contains π electron donors or π electron receptors, or both types of groups. Therefore, these CSPs are classified into three groups [21]; π-acidic (with π electron acceptor groups), π-basic (with π electron donor groups), π-acidic-basic (with π electron acceptor and donor groups), respectively.

In general, the normal phase mode has frequently been used for chiral resolution of racemic compounds on Pirkle-type CSPs [70, 71–73]. Hexane, heptane and cyclohexane are the nonpolar solvents of choice on these phases. Aliphatic alcohols may be considered as hydrogen donors and acceptors, and thus may interact at many points with the aromatic amide groups of CSPs, generating hydrogen bonds. Therefore, the addition of aliphatic alcohols improves chiral resolution, and hence the alcohols are called organic modifiers. The most commonly used alcohol is 2-propanol. However, methanol, ethanol, 1-propanol and n-butanol have also been used [74]. There are some reports which also indicate the use of dichloromethane and chloroform as organic modifiers. In addition to this, acidic and basic additives improve the chromatographic resolution. A small amount of acetic, formic or trifluoroacetic acid improves the peak shape and enantioselectivity for acidic and basic solutes. Sometimes there is a need to combine an acid and an organic amine (e.g. triethylamine) for strong basic racemic compounds [21, 69].

Ligand exchangers

Chiral ligand exchange chromatography (CLEC) is a particular form of ion exchange. CLEC was developed by Rogozhin and Davankov in 1969. Copper (II) has been used as the ligand metal ion in most of the applications of ligand exchange chromatography. It involves the reversible formation of a metal complex by coordination of substrates that can act as ligands to the metal ion; it has a wider application than the resolution of racemates [75, 76].

Ligand exchange chiral selectors involve the breaking and formation of coordinate bonds among the metal ions

of the complex, the ligands and the chiral molecules. Therefore, ligand exchange chromatography is useful for the chiral separation of molecules that contain electron-donation atoms, such between immobilized amino acids, a divalent cation, and the analyte enantiomers. In most applications, aqueous solutions of metal ions or buffers have been used as the mobile phases. The most commonly used buffers are ammonium acetate and phosphate [77–79]. The stability of such complexes is highly dependent on the transition metal used. In general, acetonitrile has been used as the organic modifier [80]; however, some reports deal with the use of methanol, ethanol, and tetrahydrofuran [80, 81–83]. The concentrations of these modifiers vary from 10% to 30 %. However, some reports have indicated the use of these organic modifiers up to 75% [82, 83]. In general, using organic modifiers optimizes the chiral resolution of highly retained solutes. These types of molecules contain amino, hydroxy, and acid groups. The pH of the mobile phase has also been recognized as one of the most important controlling factors in chiral resolution on ligand exchange chiral phases [84]. In general, the retention of all racemates increases with an increasing pH value. The selectivity of the separation was only moderately affected by pH changes, while the efficiency of the column showed a different trend, depending on the relative retentions of the racemates. The buffer concentration is also a very important aspect in the optimization of chiral resolution on these CSPs [21]. However, some other metal ions, such as nickel and zinc, have also been tested [85].

Crown ethers

The crown ethers are synthetic macrocyclic polyethers: their name derives from both the crown-like appearance of their molecular structures and their ability to crown selectively with cations. The ether oxygens that are electron donors remain in the inner wall of the crown cavity, and are surrounded by methylene groups in a collar fashion [21]. The IUPAC nomenclature for these ethers is complex, and hence trivial names are commonly used [86].

Introducing chiral moieties develops chirality in crown ethers and, hence, the developed crown ether is called chiral crown ether (CCE). The most important chiral groups used for this purpose are binaphthyl [87, 88–90], biphenanthryl [91, 92], hericene derivatives [93], tartaric acid derivatives [94], carbohydrate moiety [95], and a chiral carbon atom with a bulky group directly incorporated in the crown ring [96], aromatic bicyclo derivatives, nonane derivatives [97], hexahydrochrysese or tetrahydroindenoinden [98], and 9, 9 -spiro-bifluorene groups [99].

Aqueous mobile phases containing organic modifiers and acids have been used on these CSPs. The commonly used mobile phases are aqueous, perchloric acid and aqueous methanol containing sulfuric, trifluoroacetic or perchloric acid separately. Compounds with a higher hydrophobicity generally have longer retention times on CCE-based CSPs and, therefore, organic modifiers are used to optimize the resolution. In general, the separation is increased by an increase in methanol and a decrease in the acid concentrations. The other organic modifiers used

are ethanol, acetonitrile and THF, but methanol has been found to be the best one [21]. The application of these chiral selectors is limited. However, they can be used for the analysis of chiral molecules containing amine and amide groups [100, 101–104].

Macrocyclic antibiotics

Macrocyclic antibiotics possess several characteristics that allow them to interact with analytes and serve as chiral selectors (CSs). Only few chiral selectors offer a high degree of selectivity for numerous compounds while providing good efficiency. The macrocyclic antibiotics are the most promising in this respect and, in fact, they have already had an immediate and significant impact on the field of separations since their introduction by Daniel W. Armstrong in Pittsburgh (1994). They have a number of stereogenic centres and functional groups, allowing them to have multiple interactions with chiral molecules. They typically have molecular masses between 600 and 2200 and have numerous functional groups. They may be acidic, basic or neutral, and may have little or no UV-VIS absorbance. The macrocyclic antibiotics can interact by hydrophobic, dipole-dipole, π-π interactions, hydrogen bonding, as well as steric repulsion [2, 105, 106]. One of the more important interactions is an ionic or charge-to charge interaction. In addition to the hydrophobic moieties, these molecules possess hydrophilic groups as well as a number of ionisable groups, giving them good solubility in aqueous solution [2].

The most successful and most extensively used macrocyclic antibiotic CSs have become the glycopeptides [2, 107, 108]. Ansamycin, the polypeptide thiostrepton and the amino glycosides, fradiomycin, kanamycin and streptomycin, also have been used as CSs [105, 109].

The macrocyclic glycopeptide antibiotics include avoparcin, ristocetin A, teicoplanin, vancomycin and their derivatized analogs. By changing the glycopeptide antibiotic used, the enantioselectivity of the separations can be significantly altered. While the glycopeptide antibiotics have similar structures, they often exhibit different but complementary enantioselectivities. This suggests that the mechanism of separation is similar though not identical. Consequently, if only a partial separation is obtained using one of the glycopeptides, there is an excellent probability that a baseline or better separation may be obtained with one of the other glycopeptides [107, 109].

The macrocyclic antibiotics can be operated in the reversed phase, normal phase, and polar organic phase conditions. A variety of organic modifiers (methanol, ethanol, 2-propanol, acetonitrile, and tetrahydrofuran) have been used to alter selectivity. Hexane in combination with acetonitrile, methanol or ethanol is the most common composition of the mobile phase in the normal phase chromatography. They can be used as chiral selectors for the separation of amino acids, carboxylate compounds [110–112], and also chiral sulfoxides [113] and many other compounds due to their excellent chiral recognition capabilities. Their ability to form simultaneous polar and ionic interactions via the substituents from their multiple

chiral centres and binding sites that are located in and about the cavities of the glycopeptide's basket–like structure is responsible for recognition capabilities [112, 114].

REFERENCES

1. **Hefnawy, M. M., Aboul-Enein H. Y.:** Talanta, 2003; 61, 667-673.
2. **Ward, T. J., Farris III, A. B.:** J. Chromatogr. A, 2001; 906, 73-89.
3. **Ward, T. J.:** Anal. Chem., 1994; 66, 632 A-640 A.
4. **Aboul-Enein, H. Y., Ali, I.:** II Farmaco, 2002; 57, 513-529.
5. **Ding, G.-S., Liu, Y., Cong, R.-Z., Wang, J.-D.:** Talanta, 2004; 62, 997-1003.
6. **Lindner, W.:** (Preface to chiral Separations Volume) J. Chromatogr. A, 1994; 666, 1.
7. **Allenmark, S., Schurig, V.:** J. Mater. Chem., 1997; 7, 1955-1963.
8. **Cass, Q.B., Batigalhia, F.:** J. Chromatogr. A, 2003; 987, 445-452.
9. **Ahuja, S.:** In: Ahuja, S. Chiral separation by liquid chromatography. ACS Symposium, ACS, Washington, DC, 1991; 471, 1-26.
10. **Kallenborn, R., Hühnerfuss, H.:** Chiral Environmental Pollutants, Trace Analysis and Ecotoxicology, Springer–Verlag, Berlin, 2001; s. 3.
11. **Kondepudi, D. K., Durand, D. J.:** J. Chirality, 2001; 13, 351-356.
12. **Bailey, J., Chrysostomou, A., Hough, J. H. et al.:** Science, 1998; 281, 672-674.
13. **Schreier, P., Bernreuther, A., Huffer, M.:** Analysis of chiral organic molecules, Methodology and applications. Berlin, New York, Walter de Gruyter, 1995.
14. **Allenmark, S.:** Chromatografic enantioseparation, Methods and applications. Second edition, Ellis Horwood, 1991.
15. **Eliel, E. L., Wilen, S. H.:** Stereochemistry of organic compounds. New York, Wiley, 1994.
16. **Aboul-Enein, H. Y., Ali, I.:** Chiral Separations by Liquid Chromatography and Related Technologies. New York, Dekker, 2003.
17. **Subramanian, G. (ed.):** A Practical Approach to Chiral Separations by Liquid Chromatography. Weinheim, VCH, 1994.
18. **Aboul-Enein, H. Y., Wainer, I. W. (eds.):** The Impact of Stereochemistry on Drug Development and Use, Chemical Analysis, vol. 142. New York, Wiley, 1997.
19. **Maier, N. M., Franco, P., Lindner, W.:** J. Chromatogr. A, 2001; 906, 3-33.
20. **Stefan, R. I., van Staden, J. F., Aboul–Enein, H. Y.:** Electrochemical Sensors in Bioanalysis. New York, Dekker, 2001.
21. **Ali, I., Aboul-Enein, H. Y.:** Chiral Pollutants: Distribution, Toxicity and Analysis by Chromatography and Capillary Electrophoresis. John Wiley & Sons, Ltd. 2004.
22. **Pirkle, W. H.:** In: Ahuja, S. Chromatography and separation chemistry, Ed. ACS, Washington, D.C.,1986, s. 101.
23. **Ahuja, S.:** Chiral separation by chromatography. ACS, Washington, D.C., 2000, s. 112.
24. **Okamoto, Y.:** Chemtech., 1987; 17, 176.
25. **Okamoto, Y., Yashima, E.:** In: Hatada, K., Kitayama, T., Vogl, O. Molecular Design of Polymeric Materials. Eds. New York, Dekker, 1997; s. 731.
26. **Yashima, E., Okamoto, Y.:** In: Aboul-Enein, H. Y., Wainer, I. W. eds, The Impact of Stereochemistry on Drugs Development and Use. New York, Wiley, 1997; s. 345.
27. **Ichida, A., Shibata, T., Okamoto, Y. et al.:** Chromatographia, 1984; 19, 280.
28. **Stringham, R. W., Ye, Y. K.:** J. Chromatogr. A, 2006; 1101, 86-93.
29. **Ye, Y. K., Lord, B. S., Yin, L., Stringham, R. W.:** J. Chromatogr. A, 2002; 945,147-159.
30. **Mesplet, N., Saito,Y., Morin,P., Agrofoglio, L. A.:** J. Chromatogr. A, 2003; 983, 115-124.
31. **Cirilli, R., Costi, R., Di Santo, R. et al.:** J. Chromatogr. A, 2003; 993, 17-28.
32. **Matthijs, N., Perrin, C., Maftouh, M. et al.:** J. Chromatogr. A, 2004;1041, 119-133.
33. **Zongde, Z., Xingping,L., Xiaomei, W. et al.:** J. Biochem. Biophys. Methods, 2005; 62, 69-79.
34. **Lao, W., Gan, J.:** J. Chromatogr. A, 2006; 1117, 184-193.
35. **Sellers, J. A., Olsen, B. A., Owens, P. K., Gavin, P. F.:** J. Pharm. Biomed. Anal., 2006; 41, 1088-1094.
36. **de Veredas, V., Carpes, M. J. S., Correia, C. R. D., Santana, C.C.:** J. Chromatogr. A, 2006; 1119, 156-162.
37. **Zhao, Y., Pritts, W. A .:** J. Chromatogr. A, 2007; in press.
38. **Zhai, Z. D., Shi, Y.P., Wang, T.:** Anal. Chimica Acta, 2005; 550, 123-129.
39. **Menges, R. A., Armstrong, D. W.:** In: Ahuja, S. Chiral separation by liquid chromatography. Washington, DC, ACS, 1991; 471, 67-100.
40. **Bender, M. L., Komiyama, M.:** Cyclodextrin Chemistry, Springer–Verlag, Berlin, 1978.
41. **Han, S. M., Armstrong, D. W.:** In: Krstulovic, A. M. ed., Chiral Separations by HPLC, Ellis Horwood, Chichester, 1989; s. 208.
42. **Stalcup, A. M.:** in Subramanian, G. ed., A Practical Approach to Chiral Separations by Liquid Chromatography. Weinheim, VCH, 1994; s. 95.
43. **Armstrong, D. W., DeMond, W.:** J. Chromatogr. Sci, 1984; 22, 411-415.
44. **Armstrong, D. W., Chang, C. D., Lee, S. H.:** J. Chromatogr. 1991; 539, 83-90.
45. **Simplício, A. L., Matias, P., Gilmer, J. F., Clancy, J. M.:** J. Chromatogr. A, 2006; 1120, 89-93.
46. **Bhushan, R., Gupta, D.:** J. Chromatogr. B, 2006; 837, 133-137.
47. **Feitsma, K. G., Bosman, J., Drenth B. F. H., De Zeeuw, R. A.:** J. Chromatogr. 1985; 333, 59-68.
48. **Han, S. M.:** Biomed. Chromatogr. 1997; 11, 259-271.
49. **Pellati, F., Benvenuti, S., Melegari, M.:** J. Pharm. and Biomed. Anal., 2005; 37, 839-849.
50. **Moaddel, R., Price, G. B., Juteau, J. M. et al.:** J. Chromatogr. B, 2005; 820, 197-203.
51. **Millot, M.C.:** J. Chromatogr. B, 2003; 797, 131-159.
52. **Haginaka, J.:** J. Chromatogr. A, 2001; 906, 253-273.
53. **Allenmark, S. G.:** In: Ahuja, S. Chiral separation by liquid chromatography. ACS, Washington, DC, 1991; 471, 115-125.
54. **Russeva, V. N., Zhivkova, Z. D.:** Int. J. Pharm., 1999; 180, 69-74.
55. **Nakamura, M., Kiyohara, S., Saito, K. et al.:** J. Chromatogr. A, 1998; 822, 53-58.
56. **Hofstetter, H., Hofstetter,O., Schurig, V.:** J. Chromatogr. A, 1997; 764, 35-41.

57. **Klemm, L. H., Reed, D.:** J. Chromatogr., 1960; 3, 364-368.
58. **Klemm, L. H., Desai, K. B., Spooner, J. R.:** J. Chromatogr., 1964; 14, 297-300.
59. **Mikeš, F., Boshart, G., Gil–Av, E.:** J. Chromatogr., 1976; 122, 205-221.
60. **Pirkle, W. H., House, D. W.:** J. Org. Chem., 1979; 44, 1957-1960.
61. **Pirkle, W. H., House, D. W., Finn, J. M.:** J. Chromatogr., 1980; 192, 143-158.
62. **Pirkle, W. H., Finn, J. M.:** J. Org. Chem., 1981; 46, 2935-2938.
63. **Pirkle ,W. H., Welch, C.:** J. Org. Chem., 1984; 49, 138-140.
64. **Pirkle, W. H., Däppen, R.:** J. Chromatogr., 1987; 404, 107-115.
65. **Persson, B.-A., Andersson, S.:** J. Chromatogr. A, 2001; 906, 195-203.
66. **Pirkle, W. H., Sikkenga, D. L.:** J. Chromatogr., 1976; 123, 400-404.
67. **Pirkle, W. H., Pochapsky, T. C.:** J. Am. Chem. Soc., 1986; 108, 352-354.
68. **Pirkle, W. H., Pochapsky, T. C.:** Chem. Rev., 1989; 89, 347-362.
69. **Perrin, S. R., Pirkle, W. H.:** In: Ahuja, S. Chiral separations by liquid chromatography. Washington, DC, ACS, 1991; 471, 45-66
70. **Pirkle, W. H., Koscho, M. E.:** J. Chromatogr. A, 1997; 761, 65-70.
71. **Abu–Lafi, S., Turujman, S. A**.: J. Chromatogr. A, 1999; 855, 157-170.
72. **Pirkle, W. H.,. Koscho, M. E.:** J. Chromatogr. A, 1999; 840, 151-158.
73. **Siluveru, M., Stewart, J. T.:** J. Chromatogr. B, 1997; 690, 359-362.
74. **Petersen, P. V., Ekelund, J., Olsen, L., Ovesen, S. V.:** J. Chromatogr. A, 1997; 757, 65-71.
75. **Doury–Berthod, M., Poitrenaud, C., Tremillon, B.:** J.Chromatogr.1977; 131, 73-90.
76. **Davankov, V. A., Semechkin, A. V.:** J. Chromatogr. 1977; 141, 313-353.
77. **Grobuschek, N., Schmid, M. G. C., Tuscher, C. et al.:** J. Pharm. Biomed. Anal., 2002; 27, 599-605.
78. **Galaverna, G., Corradini, R., Dallavalle, F. et al.:** J. Chromatogr. A, 2001; 922, 151-163.
79. **Galaverna, G., Corradini, R., Dossena, A. et al.:** J. Chromatogr. A, 1998; 829, 101-113.
80. **Feibush, B., Cohen, M. J., Karger, B. L.:** J. Chromatogr. 1983; 282, 3-26.
81. **Roumeliotis, P., Unger, K. K., Kurganov, A. A., Davankov, V. A.:** J. Chromatogr. 1983; 255, 51-66.
82. **Roumeliotis, P., Kurganov, A. A., Davankov, V. A.:** J. Chromatogr.1983, 266, 439-450.
83. **Shieh, C. H., Karger, B. L., Gelber, L. R., Feibush, B.:** J. Chromatogr. 1987, 406, 343-352.
84. **Remelli, M., Fornasari, P., Dondi, F., Pulidori, F.:** Chromatographia 1993; 37, 23-30.
85. **Zolotarev, Y. A., Myasoedov, N. F., Penkina, V. I. et al.:** J. Chromatogr. 1981; 207, 63-68.
86. **Pedersen, C. J.:** J. Am. Chem. Soc. 1967; 89, 7017.
87. **Kyba, E. P., Timko, J. M., Kaplan, J. L. et al.:** J. Am. Chem. Soc. 1978; 100, 4555-4568.
88. **Peacock, S. C., Domeier, L. A., Gaeta, F. C. A. et al.:** J. Am. Chem. Soc. 1978; 100, 8190-8202.
89. **Peacock, S. C., Walba, D. M., Gaeta, F. C. A. et al.:** J. Am. Chem. Soc. 1980; 102, 2043.
90. **Linggenfelter, D. S., Helgeson, R. C., Cram, D. J.:** J. Org. Chem. 1981; 46, 393-406.
91. **Yamamoto, K., Yumioka, H., Okamoto, Y., Chikamatsu, H.:** J. Chem. Soc. Chem. Commun., 1987; s. 168.
92. **Yamamoto, K., Noda, K., Okamoto, Y.:** J. Chem. Soc. Chem. Commun., 1985; 1065.
93. **Nakazaki, M., Yamamoto, K., Ikeda, T. et al.:** J. Chem. Soc. Chem. Commun., 1983; 787.
94. **Behr, J. P., Girodeau, J. M., Hayward, R. C. et al.:** Helv. Chim. Acta, 1980; 63, 2096-2111.
95. **Gehin, D., Cesare, P. D., Gross, B.:** J. Org. Chem., 1986; 51, 1906-1908.
96. **Davidson, R. B., Bradshaw, J. S., Jones, B. A. et al.:** J. Org. Chem. 1984; 49, 353-357.
97. **Naemura, K., Fukunaga, R.:** Chem. Lett., 1985; 1651.
98. **Naemura, K., Komatsu, M., Adachi, K., Chikamatsu, H.:** J. Chem. Soc. Chem. Commun., 1986; 1675.
99. **Thoma, A. P., Viviani–Nauer, A., Schellenberg, K. H. et al.:** Helv. Chim. Acta, 1979, 62, 2303.
100. **Steffeck, R. J., Zelechonok, Y., Gahm, K.H.:** J. Chromatogr. A, 2002; 947, 301-305.
101. **Hyuna, M. H., Hana, S. Ch., Lipshutzb, B. H. et al.:** J. Chromatogr. A, 2002; 959, 75-83.
102. **Hyun, M. H., Han, S. Ch.:** J. Biochem. Biophys. Methods, 2002; 54, 235-243.
103. **Seyhan, S., Turgut, Y., Merdivana, M., Hoşgören, H.:** Tetrahedron: Asymmetry, 2006; 17, 1700-1704.
104. **Farkas, V., Tóth, T., Orosz, G. et al.:** Tetrahedron: Asymmetry, 2006; 17, 1883-1889.
105. **Armstrong, D. W., Nair, U. B.:** Electrophoresis, 1997; 18, 2331-2342.
106. **Ward, T. J., Oswald, T. M.:** J. Chromatogr. A, 1997; 792, 309-325.
107. **Gasper, M. P., Berthod, A., Nair, U. B., Armstrong, D. W.:** Anal.Chem.1996; 68, 2501-2514.
108. **Ward, T. J., Dann III, C., Blaylock, A.:** J. Chromatogr. A, 1995; 715, 337-344.
109. **Verleysen, K., Sandra, P.:** Electrophoresis, 1998; 19, 2798-2833.
110. **Beesley, T. E., Scott, R. P. W. (eds.):** Chiral Chromatography. Chichester, NS Wiley, 1998; s. 242.
111. **Lämmerhofer, M., Lindner, W.:** In: Valkó, K. (ed.), Separation Methods in Drug Synthesis and Purification. Amsterdam, Elsevier, 2000; s. 381.
112. **Jandera, P., Bačkovská, V., Felinger, A.:** J. Chromatogr. A, 2001; 919, 67-77.
113. **Berthod, A., Xiao, T.L., Liu, Y. et al.:** J. Chromatogr. A, 2002; 955, 53-69.
114. **Armstrong, D. W., Liu, Y., Ekborg–Ott, K. H.:** Chirality, 1995; 7, 474-497.