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Chiral Separation of Some Classes of Pesticides by HPLC Method

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2.1
Introduction

Pesticides are one of the most notorious organic pollutants in our environment. According to pests, they are classified as insecticide, fungicide, herbicide, algaecide, avicide, bactericide, miticide, molluscicide, nematicide, piscicide, and rodenticide. The three major classes of pesticides are insecticide, herbicide, and fungicide, while the rest are classified as miscellaneous pesticides. About 1693 pesticides are available, and the majority of pesticides are organic chemicals, while some are inorganic or biological species [1]. These pesticides control the insect by killing it or by changing its behavior, and are delivered through systems such as spraying, baits, slow-release diffusion, and so on. Insecticides are classified as organochlorine compounds (biphenyl aliphatic, hexachlorocyclohexane, cyclodienes, and polychloroterpenes), organophosphate compounds – esters of phosphorus (phosphates, phosphonates, phosphorothioates, phosphorodithioates, and phosphoramidates), organosulfur compounds that contain two phenyl rings with sulfur atoms, carbamates, formamidines, dinitrophenols, organotins, pyrethroids (first-, second-, third-, and fourth-generation pyrethroids), nicotinoids, spynosins, fiproles or phenyl-pyrazoles, pyrroles, pyrazoles, pyridazinones, quinazolines, benzoyl urea, botanicals (pyrethrum, nicotine, rotenone, limonene, or *d*-limonene, and neem), synergists or activators, antibiotics, fumigants, insect repellents, inorganics, miscellaneous classes of insecticides (methoxyacrylates, naphthoquinones, nereistoxin analogs, pyridine azomethine, pyrimidinamines, and tetronic acids), and miscellaneous compounds of insecticides: etoxazole, pyridalyl, amidoflumet, pyriproxyfen, buprofezin, and tebufenozide [2].

Fungicides are useful in controlling fungal diseases by specifically inhibiting or killing the fungus that causes the disease. Fungicides are classified on the basis of their chemical compositions that include benzimidazole, dicarboximide, imidazole, piperazine, triazole, phenylamide, oxathiin, anilinopyrimidine, strobilurin, phenylpyrrole, chloronitrobenzene, thiadiazole, cinnamic acid, hydroxyanilide, streptomyces, polyoxin, benzothiadiazole, phosphonate, dithiocarbamate, chloroalkythiols, chloronitrile, phenylpyridinamine, cyanoacetamide oxime, carbamate,

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aldehyde, mineral oils, and some inorganics [3]. Herbicides (weed killers) are useful for weed control and are classified as phenoxy compounds, phenylacetic acid, benzoic acid, phthalic acid, *N*-1-napthylphthalamic acid, aliphatic acid, substituted phenols, heterocyclic nitrogen derivatives, aliphatic organic nitrogen derivatives, carbamate, metal organic and inorganic salts, and hydrocarbons or oils [4].

Among these pesticides about 28% (482) are chiral in nature, of which 149 are insecticides, 141 are herbicides, 97 are fungicides, and 95 are miscellaneous chiral pesticides [1]. These pesticides are useful for food production since they reduce the rate of diseases in crops. However, pesticides are also harmful both to the environment and to humans. Therefore, it is desirable to reduce the consumption of pesticides to a minimum. Only one enantiomer is biologically active toward the target organisms, while the other enantiomer has less impact (less active), but it may cause adverse effects on some nontarget organisms and, thus, it becomes an unwanted burden for the environment and becomes a potential health hazard. Accordingly, we can reduce the use of pesticides on the basis of chirality [5,6]. Moreover, the enantioselective behavior of chiral pesticides and physiological changes of plants may alter the food chain and become beneficial to the ecological system. Moreover, degradation products of achiral pesticides may be chiral and toxic. The enantiomers can exhibit significant differences both in their biological activity and toxicity and in their environmental behavior [7–14]. Therefore, it is important to investigate the actual impact of chiral pesticides on the environment. In spite of this, a majority of chiral pesticides are being marketed and released into the environment as racemates.

Consequently, there is an urgent need to develop analytical methods to determine the optical purity and the enantiomeric resolution of chiral pesticides. Several analytical methods have been used to assess the enantiomeric purity of different classes of pesticides. High-performance liquid chromatography (HPLC) has achieved a good reputation for chiral analysis of pesticides due to the availability of several chiral stationary phases (CSPs), its high speed, sensitivity, and reproducibility. Although the CSPs are generally expensive, they are reliable and provide robust analytical results.

A variety of mobile phases, including normal, reversed, and new polar organic phases, are used in HPLC. About 80% of the enantiomeric separations of drugs, pharmaceuticals, and pesticides have been carried out by HPLC [15–22]. As a result, a wide range of applications of HPLC in the chiral resolution of pesticides are presented in this chapter using normal and reversed chiral stationary phases. The mechanism of chiral separation of pesticides using various chiral stationary phases will be discussed.

2.2 Mechanism of Chiral Separation

Several chiral stationary phases (CSPs) have been used for the enantiomeric separation of pesticides that include polysaccharides, cyclodextrins, macrocyclic

glycopeptide antibiotics, proteins, crown ethers, ligand exchangers, Pirkle's types, and several others [15,23]. Yet, the chiral recognition mechanism(s) at the molecular level on these CSPs is still not fully investigated. Pfeiffer [24] in 1956 explained the three-point model for chiral recognition mechanisms. Furthermore, this model was explained in more detail by Pirkle and Pochapsky [25]. According to the model in question, a minimum of three interactions between the CSP and at least one of the enantiomers play an important role in chiral separation, and at least one of these interactions is stereochemically dependent, that is, one interaction involves the stereogenic center with the CSP. This three-point model is not applicable for every chiral species. Groombridge *et al.* [26] postulated a fourpoint model for chiral recognition on some protein CSPs that depends on the formation of transient diastereoisomeric complexes between the enantiomers and the CSP that possess different physical and chemical properties and, as a result, the said enantiomers are resolved at different retention times. Every chiral stationary phase has a different chiral recognition mechanism. However, chiral recognition processes on polysaccharides, cyclodextrins (CDs), macrocyclic glycopeptide antibiotics, proteins, and chiral crown ether-based CSPs are more or less similar. The chiral grooves on polysaccharides, the cavities on CDs, the baskets on macrocyclic glycopeptide antibiotics, the bridges and the loops on proteins, and the cavities on chiral crown ether-based CSPs all provide a chiral environment for enantiomers. Two enantiomers get fitted to different extents and, hence, elute at different retention times. The differences in the stabilities of the enantiomers on these CSPs are due to their different bondings and interactions, the most important of which are hydrogen bonding, dipole-induced dipole interactions, π–π complexation, inclusion complexation, anionic and cationic bonding, and van der Waals forces [15–21]. Steric effects also play a crucial role in the chiral resolution of racemates. Different binding energies of the transient diastereoisomeric complexes result from the various interactions mentioned above. Pirkle-type CSPs contain a chiral aromatic ring; therefore, the formation of a π–π charge transfer diastereoisomeric complexes of the enantiomers (with the aromatic group) with a CSP is considered to be essential. In view of these facts, the π acidic CSPs are suitable for chiral resolution of π donor solutes, and vice versa. However, the newly developed CSPs that contain both π acidic and π basic groups are suitable for the chiral resolution of both types of solutes, that is, π donor and π acceptor analytes. In brief, Pirkle-type CSPs contain a chiral moiety that provides a chiral environment for the enantiomers. Therefore, the enantiomers fit differently to this chiral moiety (due to their different spatial configurations). Accordingly, the two enantiomers form diastereoisomeric complexes that have different physical and chemical properties, along with different binding energies. Therefore, the two enantiomers elute at different retention times with the flow rate of the mobile phase and, hence, chiral separation occurs.

On ligand-exchange CSPs, chiral resolution occurs due to the exchange of chiral ligands and enantiomers on specific metal ions through coordinate bonds. The two enantiomers have different exchange capacities because of the

stereospecific nature of the ligand-exchange process and, hence, chiral resolution takes place. Rogozhin and Davankov [27–29] suggested a theoretical model for the mechanism of chiral resolution on these CSPs. In this model, the enantiomers are coordinated to metal ion in different ways depending on their interactions with ligands bonded to the stationary phases that act as the chiral selectors. The authors explained that chiral resolution occurs because of the different bonding along with the steric effects that result in the formation of the transient diastereoisomeric complexes by the two enantiomers. These transient diastereoisomeric complexes are stabilized at different magnitudes by dipole–dipole interactions, hydrogen bonding, van der Waals forces, and steric effects, and elute at different retention times. A general graphical representation of the chiral resolution mechanism of racemates on the aforementioned CSPs is shown in Figure 2.1.

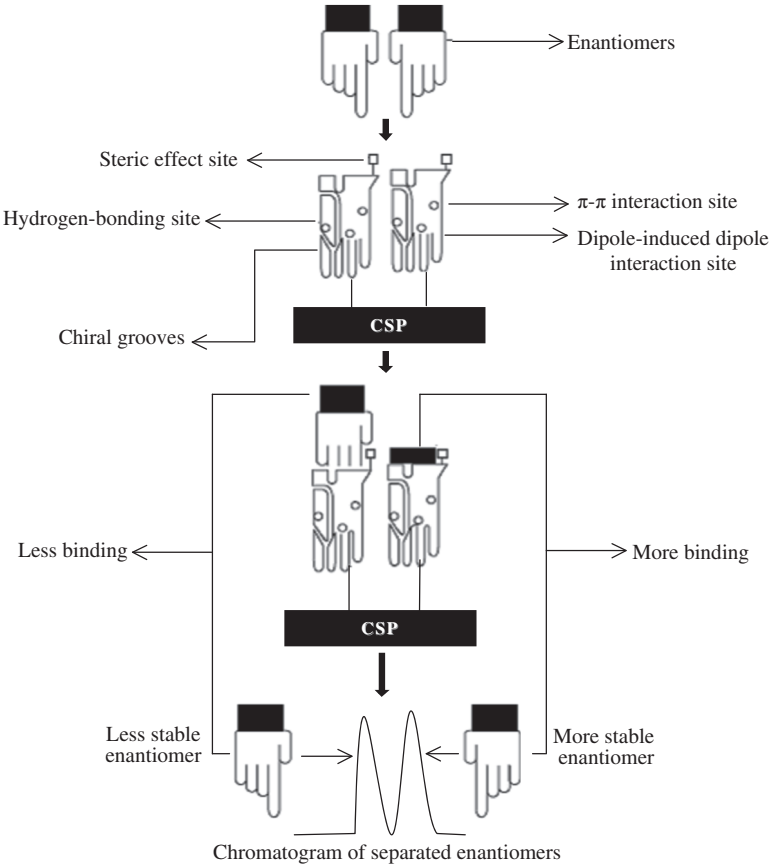


Figure 2.1 Schematic diagram of chiral resolution mechanism.

2.3
Columns and Eluents

A variety of chiral stationary phases have been developed for the resolution of chiral pesticides. The most important classes of chiral selectors have been explained above. These chiral selectors are available in the form of columns and capillaries, and are marketed under different trade names as shown in Table 2.1. The development of the chiral stationary phases enhanced the utility of high-performance liquid chromatographic technique. These CSPs have been used frequently and successfully in the chiral resolution of many drugs, pharmaceuticals, and other environmental pollutants. Therefore, they may also be used for the enantiomeric separation of chiral pesticides and some reports on the chiral separation of pesticides using the above-mentioned CSPs are to be found in the literature. In a significant proportion of resolutions of chiral pesticides, using these chiral stationary phases, a wide range of mobile phases have been used as eluents. Different compositions of eluents for chiral separation depend on the chiral stationary phase. For the normal-phase mode, various compositions of organic modifiers can be employed, whereas for the reversed phase, polar organic solvents with water and buffer are used. The different optimizing conditions for the chiral resolution include the composition of a mobile phase, its pH, the temperature, the amount injected into the column, the flow rate, and the detection mode.

In the case of polysaccharide chiral columns, for normal-phase chromatography, pure ethanol or 2-propanol as an eluent is recommended. In order to decrease the polarity of the mobile phase and increase the retention times of the enantiomers, hexane, cyclohexane, pentane, and heptane are used as the main constituents of the mobile phase. However, other alcohols are also used in the mobile phase. Normally, if pure ethanol or 2-propanol is not suitable for the mobile phase, hexane, 2-propanol, or ethanol with a ratio of 80:20 v/v is used in the mobile phase and the change in the mobile-phase composition is introduced on the basis of observations. Finally, the optimization of the chiral resolution is carried out by adding small amounts of amines or acids (0.1–1.0%). Similarly, the chiral resolution on polysaccharide-based CSPs in the reversed phase mode is carried out using the aqueous mobile phases, and the selection of a mobile phase depends on the solubility and the properties of pesticides to be analyzed. The choice of a mobile phase in the reversed phase mode is very limited. Water is used as the main constituent of the mobile phases. The modifiers used are acetonitrile, methanol, and ethanol. The optimization of the chiral resolution is carried out by adding small quantities of amines or acids (0.1–1.0%). Some of the resolutions are pH dependent and require a constant pH of a mobile phase. Under such conditions, in general, the resolution is not reproducible using mobile phases such as water–acetonitrile or water–methanol and, therefore, a buffer with some organic modifiers (acetonitrile, methanol) have been used as the mobile phase. The optimization of the resolution is carried out by adjusting

Table 2.1 Various normal- and reversed phase chiral columns and their trade names.

Columns	Trade name
Polysaccharide-based CSPs	
Chiralcel OB, Chiralcel OB-H, Chiralcel OJ, Chiralcel OJ-R, Chiralcel CMB, Chiralcel OC, Chiralcel OD, Chiralcel OD-H, Chiralcel OD-R, Chiralcel OF, Chiralcel OD-RH, Chiralcel OG, Chiralcel OA, Chiralcel CTA, Chiralcel OK, Chiralpak AD, Chiralpak AD-R, Chiralpak AR Chiralpak AD-RH, and Chiralpak AS	Daicel Chemical Industries, Tokyo, Japan
Cyclodextrin-based CSPs	
Cyclobond I, II, and III; Cyclobond AC, RN, SN; ApHpera ACD and BCD	Advanced Separation Technologies, Whippany, NJ, USA.
Nucleodex β-OH, Nucleodex β-PM	Macherey-Nagel, Duren, Germany.
ORpak CD-HQ, and Orpak CDB-453 HQ, ORpak CDBS-453, ORpak CDA-453 HQ, and ORpak CDC-453 HQ	Showa Denko, Kanagawa, Japan
Keystone β-OH and Keystone β-PM	Thermo Hypersil, Bellefonte, PA, USA
B-Cyclose-6-OH	Chiral Separations, La Frenaye, France
YMC Chiral cyclodextrin BR, YMC Chiral NEA (R), and YMC Chiral NEA (S)	YMC, Kyoto, Japan
Macrocyclicglycopeptide antibiotic-based CSPs	
Chirobiotic R, Chirobiotic T, Chirobiotic V, Chirobiotic TAG, and Chirobioticmodified V	Advanced Separation Technologies, Inc., Whippany, NJ, USA
Protein-based CSPs	
Chiral AGP, Chiral HSA, and Chiral CBH	Advanced Separation Technologies, Inc., Whippany, NJ, USA
Chiral AGP, Chiral CBH, and Chiral HAS	Chrom Tech, Ltd, Cheshire, UK
Resolvosil BSA-7 and Resolvosil BSA-7PX	Macherey-Nagel, Düren, Germany
Chiral AGP, Chiral CBH, and Chiral HAS	Regis Technologies, Morton Grove, IL, USA
AFpak ABA-894	Showa Denko, Kanagawa, Japan
Keystone HSA and Keystone BAS	Thermo Hypersil, Bellefonte, PA,USA
TSKgelEnantio L1 and TSK gel Enantio-OVM	Tosoh, Tokyo, Japan
EnantioPac,	LKB Pharmacia, Bromma, Sweden
Bioptic AV-1	GL Sciences, Tokyo, Japan
Ultron ES-BSA, Ultron ES-OVM Column, Ultron ES-OGP Column, and Ultron ES-Pepsin	Shinwa Chemical Industries, Kyoto, Japan
Crown ether-based CSPs	
Crownpak CR	Chiral Technologies, Inc., Exton, PA, USA

	Separations Kasunigaseki-Chrome, Tokyo, Japan Daicel Chemical Industries, Tokyo, Japan
Opticrown RCA	USmac Corporation, Winnetka, Glenview, IL, USA
Chiralhyun CR-1	K-MAC (Korea Materials & Analysis Corp.), South Korea
Chirosil CH-RCA	Restech Corporation, Daedeok, Daejon, South Korea
Ligand exchange-based CSPs	
Chirosolve	JPS Chemie, Switzerland
Chiralpak WH, Chiralpak WM, Chiralpak WE, and Chiralpak MA	Separations Kasunigaseki-Chrome, Tokyo, Japan
Nucleosil Chiral-1	Macherey-Nagel, Düren, Germany
Phenylglycine and leucine Chirex types	Regis Technologies, Morton Grove, IL, USA
Orpak CRX-853	Showa Denko, Kanagawa, Japan
Pirkle-type CSPs	
Opticrown chiralhyun-Leu-1 and Opticrown Chiralhyun-PG-1	Usmac Corporation, Glenview, IL, USA
Whelk-O 1, Whelk-O 2, leucine, phenylglycine, β-Gem 1, α-Burke 1, α-Burke 2, Pirkle 1-J, naphthylleucine, Ulmo, and Dach	Regis Technologies, Morton Grove, IL, USA
Nucleosil Chiral-2	Macherey-Nagel, Düren, Germany
Sumichiral OA	Sumika Chemical Analysis Service, Konohana-ku Osaka, Japan
Kromasil Chiral TBB and Kromasil Chiral DMB	Eka Chemicals Separation Products, Bohus, Sweden
Chirex type I	Phenomenex, Torrance, CA, USA
Chiris series	IRIS Technologies, Lawrence, KS, USA

the pH values of the buffers and the amounts of the organic modifiers. The most commonly used buffers are perchlorate, acetate, and phosphate.

For the cyclodextrin-based columns, chiral resolutions have been carried out using aqueous mobile phases. Buffers of different concentrations and pH values have been used for this purpose. Triethylammonium acetate (TEAA), phosphate, citrate, and acetate are among the most commonly used buffers [30–33]. Phosphate buffers, such as sodium, potassium, and ammonium phosphate are commonly used. The stability constant of the complexes decreases due to the addition of the organic solvents and, hence, the organic modifiers are used to optimize the chiral resolution. The most commonly used organic solvents are methanol and acetonitrile. Acetonitrile is stronger than methanol. Some other organic modifiers such as ethanol, 2-propanol, 1-propanol, *n*-butanol,

tetrahydrofuran, and dimethylformamide have also been used for the optimization of the chiral resolution on CD-based CSPs [30,31,34,35]. The effects of the type and concentration of these organic modifiers vary from one analyte to another. Hence, it is very difficult to predict the best strategy for their use as organic modifiers. Sometimes, the use of highly concentrated buffers under the reversed phase mode decreases the lifetime and the efficiency of a column. Therefore, the use of an alternative mobile phase, that is, the normal phase, is advantageous for the chiral resolution on these phases. The most commonly used solvents in the normal-phase mode are hexane, cyclohexane, and heptane. However, some other solvents, such as dichloromethane, acetone, 1-propanol, 2-propanol, ethyl acetate, ethanol, and chloroform, have also been used as the components of the mobile phase. The concentration of the buffer is a very important aspect of the chiral resolution on these phases under the reversed phase mode. The addition of salts to the reversed mobile phase has been found to improve the chiral resolution [33].

The macrocyclic glycopeptide antibiotic columns may be used in normal, reversed, and new polar ionic phase modes. Due to the complex structure of these antibiotics, most of them function equally well in reversed, normal, and modified polar ionic phases. All three modes generally show different selectivity with different analytes. In normal-phase chromatography, the most commonly used solvents are typically hexane, ethanol, and methanol. The optimization of chiral resolution is achieved by adding some other organic acids and bases, such as acetic acid, trifluoroacetic acid (TFA), diethylamine (DEA), or triethylamine (TEA) [36,37]. In the reversed phase mode, buffers are mostly used as mobile phases with small quantities of organic modifiers. The use of buffers as mobile phases has increased the efficiency of the resolution. Ammonium nitrate, triethylammonium acetate (TEAA), and sodium citrate buffers have been used very successfully. A variety of organic modifiers have been used to alter selectivity [38–40] and acetonitrile, methanol, ethanol, 2-propanol, and tetrahydrofuran (THF) have shown good selectivity for various analytes. In the reversed phase mode, the quantities of organic modifiers are typically low, usually on the order of 10–20%. The typical starting composition of a mobile phase is an organic modifier buffer (pH 4.0–7.0) (10:90 v/v). The use of alcohols as organic modifiers generally requires higher starting concentrations, for example, 20% for comparable retention when acetonitrile or tetrahydrofuran is used with a starting concentration of 10%. The effects of the organic solvents on the enantioselectivities also depend on the type of the antibiotic. In fact, better recognition is obtained at lower buffer pH values or close to the isoelectric point of the antibiotic, especially for vancomycin. When vancomycin CSP column is used, the low concentration of organic solvents does not significantly influence the separation, while the enantioresolution is improved for some compounds using ristocetin A and teicoplanin CSPs even when using low organic modifier concentrations [41]. The effects of organic modifiers on the chiral resolution vary from racemate to racemate [42]. The simplified approach has proven to be very effective for the resolution of a broad spectrum of racemates. The first issue to consider here is

the structure of the analytes. If a compound has more than one functional group that is capable of interacting with a stationary phase and at least one of these groups is on or near the stereogenic center, then the first choice for a mobile phase would be the new polar ionic phase. This is due to the presence of strong polar groups in macrocyclic peptides. Thus, it is possible to convert an original mobile phase to 100% methanol, with the acid/base added to improve the selectivity. The key factor in obtaining a complete resolution still depends on the ratio of acid to base. The actual concentrations of acid and base affect the retention. Therefore, starting with a ratio of 1:1 v/v, some selectivity is typically observed. This is then followed by using ratios of 1:2 v/v and 2:1v/v to monitor change in the resolution that indicates a trend. If an analyte is eluting too quickly, the acid/base concentration is reduced. Conversely, if an analyte is retained too well, the acid/base concentration is increased. The parameters of the concentrations are between 1 and 0.01%. Above 1%, an analyte is too polar and indicates a typical reversed phase system while, when below 0.01%, it indicates a normal phase system. Both trifluoroacetic acid (TFA) and acetic acid have been used as the acid components with ammonium hydroxide and triethylamine as a base. For an analyte/pesticide that has only one functional group, or for the reason of solubility, typical normal-phase solvents (hexane/ethanol) or reversed phase solvents (THF/buffer) are employed. The pH value is an important controlling factor for the enantiomeric resolution in reversed and new polar ionic phases. In general, buffers are used as mobile phases to control a pH in HPLC. The pH value of the buffers ranges from 4.0 to 7.0 in the reversed phase system. It has been observed that when a pH value increases, the values of R_s, k, and α decrease. Therefore, the safest and the most suitable pH values in the reversed phase systems vary from a pH of 4.0 to a pH of 7.0 [38,43].

The protein-based chiral columns were mostly used under a reversed phase, that is, the aqueous mobile phases are frequently used. Buffers of different concentrations and pH values are mostly used for the chiral resolution on these CSPs. The most commonly used buffers were phosphate and borate that were used in the concentration range of 20–100 mM with 2.5–8.0 pH range. However, as with all silica-based CSPs, a prolonged use of an alkaline pH buffer (> 8.5) is not advisable. On the other hand, at a lower pH, irreversible changes are possible in the cross-linked protein phases and, hence, the use of buffers with low pH values for long periods of time is not recommended. Therefore, a buffer that ranges from pH 3.0 to pH 7.0 should be chosen. A pH of 4.5 ammonium acetate buffer may be useful. For the mobile-phase development, any buffer (50 mM, pH 7.0) can be used and the optimization is carried out by changing the concentration and the pH value. The successful use of the organic solvents in the optimization of the chiral resolution on these CSPs has been reported: the hydrophobic interactions are affected by the use of these solvents, the most important of which are methanol, ethanol, 1-propanol, 2-propanol, acetonitrile, and THF. These organic modifiers have been used in the range of 1–10%. Caution must be exercised when using these organic modifiers as they can denature the protein. However, the high concentrations of methanol and acetonitrile have

been used on some of the cross-linked protein CSPs. The selection of these organic modifiers depends on the structure of the racemic compounds and the CSP used. In some cases, charged modifiers such as hexanoic acid, octanoic acid, and quaternary ammonium compounds have also been used for the optimum chiral resolution [44,45].

The aqueous mobile phases containing organic modifiers and acids have been used on the chiral crown ethers (CCEs) based CSPs. In all applications, the aqueous and acidic mobile phases are used, and the most 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 [46]. This optimization is carried out by adjusting the amounts of methanol, sulfuric acid, and perchloric acid separately. In general, the separation is enhanced 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 [47–49]. In addition to the composition of the mobile phase, the effects of other parameters, such as the temperature, the flow rate, the pH value, and the structure of the analytes, have also been studied, but only a few reports are available in the literature. It has been observed that, in general, lowering the temperature results in a better resolution. The flow rate may be used to optimize the chiral resolution of pesticides on these CSPs. Since all of the mobile phases are acidic in nature, the effect of the pH on chiral resolution is not significant.

Ligand exchange columns have been used for the chiral resolution of racemic compounds containing electron-donating atoms and, therefore, its application is confined. In most cases, buffers, sometimes containing organic modifiers, have been used as mobile phase. Therefore, the optimization has been carried out by controlling the compositions of the mobile phases. There are two strategies for the development and the use of the mobile phases on these CSPs. With a CSP that has only a chiral ligand, an aqueous mobile phase containing a suitable concentration of metal ion is used while in the case of a CSP that contains a metal ion complex as a chiral ligand, a mobile phase without a metal ion is used. In the majority of the applications, aqueous solutions of metal ions or buffers have been used as mobile phases. The most commonly used buffers are ammonium acetate and phosphate. However, the use of a phosphate buffer is avoided if a metal ion is being used as the mobile phase additive in order to prevent a complex formation between the metal ion and the phosphate that may block the column. A literature search indicates that these buffers (20–50 mM) have frequently been used for successful chiral resolution, but in some instances, organic modifiers have also been used to improve the resolution. In general, acetonitrile has been used as an organic modifier [50]; however, some reports also deal with the use of methanol, ethanol, and tetrahydrofuran [51–54]. The concentrations of these modifiers vary from 10 to 30%. However, some reports have indicated the use of these organic modifiers with a concentration up to 75% [53,54]. In

general, the chiral resolution of highly retained solutes is optimized using organic modifiers. Basically, the organic modifiers reduce the hydrophobic interactions that results in an improved resolution. The pH of the mobile phase has also been recognized as one of the most important controlling factors in the chiral resolution on ligand-exchange chiral phases [55]. The retention and the selectivity of the enantiomeric resolution have also been investigated with respect to the metal ion concentrations on these CSPs. The chiral resolution on CSPs containing only chiral ligands has been carried out using different concentrations of metal ions in the mobile phase.

A normal phase mode has frequently been used for the chiral resolution of racemic compounds on Pirkle-type CSPs. Hexane, heptane, and cyclohexane are the preferred nonpolar solvents on these chiral stationary phases. Aliphatic alcohols may be considered hydrogen donors and acceptors and, thus, may interact at many points with the aromatic amide groups of CSPs generating the hydrogen bonds. Therefore, the addition of aliphatic alcohols improves the chiral resolution. Hence, these 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. Some reports have also indicated the use of dichloromethane and chloroform as organic modifiers with hexane. 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.

There are reports available that deal with the reversed phase eluents, but a prolonged use of the reversed mobile phase is not recommended. With the development of new, more stable CSPs, the use of the reversed mobile-phase mode has become possible.

2.4 Separation of Enantiomers of Pesticides by HPLC in Normal and Reversed Phase Modes

High-performance liquid chromatography is the most popular and most widely applicable technology in the field of chiral analysis of pesticides due to the availability of a large number of chiral stationary phases in the form of normal- and reversed phase modes. Over the course of time, various liquid chromatographic approaches have been developed and applied in this field. HPLC remains the most suitable modality as a result of its advantages such as its high speed, sensitivity, and reproducibility. A variety of mobile phases, including normal, reversed, and new polar organic phases, are used in HPLC. About 80% of the chiral resolutions of pharmaceuticals, drugs, agrochemicals, and other compounds have been carried out using HPLC [15–20]. HPLC has also been used for the chiral separation of pesticides [21,22]. In spite of a variety of CSPs that are available for HPLC, they have not been used very frequently in the analysis of chiral environmental pollutants. This is due to the fact that some

organochlorine pollutants are transparent to UV radiation and, hence, the most popular UV detectors cannot be used in HPLC for detecting such xenobiotics. However, HPLC can be used jointly with MS, polarimetry, and other optical detection techniques for the chiral resolution of such types of pollutants. Moreover, a large number of reports are available for the chiral resolution of some UV-absorbing pesticides by HPLC in normal- and reversed phase modes.

2.4.1
Enantiomeric Separation of Pesticides in a Normal-Phase Mode

The enantiomeric separation of pesticides has been carried out using various types of chiral stationary phases under normal-phase conditions. Among all chiral stationary phases, polysaccharide-based CSPs are the most popular CSPs because of their versatility, durability, and loading capacity. They are effective not only under normal-phase conditions but also under reversed phase conditions when the appropriate mobile phases are used. The majority of the polysaccharide-based CSPs employed have been cellulose- and amylose-based polysaccharide columns [56,57]. Thus, Caccamese and Principato [58] separated the enantiomers of vincamine alkaloids using the Chiralpak AD column with the hexane-2-propanol and hexane-ethanol as mobile phases separately. Ellington *et al.* [59] described the successful separations of the enantiomers of various organophosphorus pesticides (crotoxyphos, dialifor, fonofos, fenamiphos, fensulfothion, isofenphos, malathion, methamidophos, profenofos, crufomate, prothiophos, and trichloronate) using Chiralpak AD, Chiralpak AS, Chiralcel OD, Chiralcel OJ, and Chiralcel OG chiral columns with different mixtures of heptane and ethanol as eluting solvents. They also studied the effects of concentrations of ethanol on the chiral resolution of organophosphorus pesticides and reported poor separation of the enantiomers of fenamiphos, fensulfothion, isofenphos, profenofos, crufomate, and trichloronate pesticides at the higher concentrations of ethanol. The effects of temperature on the chiral resolution of organophosphorus pesticides had also been reported and the maximum chiral resolution was achieved at low temperatures. The effects of temperature (from 20–60 °C) on the chiral resolution of fensulfothion on Chiralcel OJ column is shown in Figure 2.2. It may be concluded from this figure that chiral resolution is enhanced at lower temperatures and reaches its maximum at 20 °C. On the other hand, the retention times increase when the temperature decreases. Ali and Aboul-Enein [60] studied the effects of various polysaccharide chiral stationary phases on the chiral resolution of *o,p*-DDT and *o,p*-DDD on Chiralpak AD-R, Chiralcel OD-R, and Chiralcel OJ-R. The results of these findings are presented in Table 2.2, which shows that the best resolution of these pesticides has been obtained on Chiralpak AD-R under the normal-phase mode. Li *et al.* [61] developed a fast and precise HPLC method for the chiral resolution of phenthoate in soil samples. The authors used Chiralcel OD column for the chiral resolution with hexane-2-propanol (100:0.8, v/v) as the mobile phase. Aboul-Enein and Ali [62] reported that the chiral resolution on the polysaccharide-

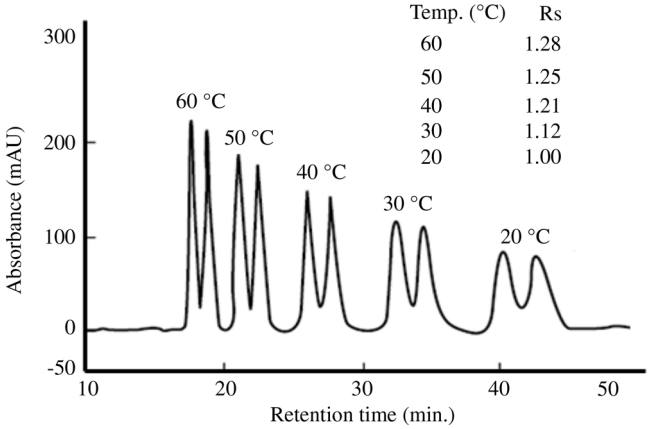


Figure 2.2 Effect of temperature on chiral separation of fensulfothion with Chiralcel OJ column [58].

based CSPs is pH dependent under the normal-phase mode. Only a partial resolution of certain antifungal agents was achieved at lower pH, while the resolution improved by increasing the pH using the triethylamine on amylose and cellulose chiral columns. Champion *et al.* [63] performed the enantiomeric separation of five polychlorinated compounds (*trans*-chlordane, *cis*-chlordane, heptachlor, heptachlor epoxide, and α-HCH) with different polysaccharide CSPs in the normal phase mode and the values of chromatographic parameters measured are shown in Table 2.2. As seen in Table 2.2, baseline separation was achieved for the enantiomers of *trans*-chlordane, *cis*-chlordane, heptachlor on Chiralcel OD column, α-HCH on Chiralcel OJ column, and heptachlor epoxide on Chiralpak AD column. The effects of the concentration of isopropanol (IPA) on the chiral resolution of *cis*-chlordane, *trans*-chlordane on Chiralcel OD column are shown in Figure 2.3. It may be concluded that a concentration of 0% of isopropanol is the most appropriate one as it enables the maximum resolution of *cis*- and *trans*-chlordane on Chiralcel OD column. Wang *et al.* [64] studied the effect of alcohols (ethanol, *n*-propanol, isopropanol, isobutanol, and *n*-butanol) on the resolutions of fipronil, isocarbophos, and carfentrazone-ethyl pesticides on the cellulose-tris(3,5-dimethylphenylcarbamate) chiral stationary phase. The concentration range for each alcohol has been 2–20%; however, in the case of carfentrazone-ethyl, it was 0.1–20% as shown in Table 2.2. The best results for the resolution of fipronil and isocarbophos was obtained using 5% isobutanol and 5% isopropanol, respectively. At the same time, the best separation of carfentrazone-ethyl was obtained with 0.5% isopropanol, while no resolution was observed with ethanol, *n*-propanol, *n*-butanol, and isobutanol from 20 to 2 %. Liu, Gan, and Qin [65] carried out the enantiomeric resolution of synthetic pyrethroid (bifenthrin, permethrin, cypermethrin, and cyfluthrin) on Sumichiral OA-2500-I and the two chained Chirex 00G-3019-DO columns with hexane-1,

Table 2.2 Chiral separation of pesticides on different columns with normal and reversed mobile-phase conditions.

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
trans-Chlordane	Chiralcel-OD	Hexane/isopropanol (99–1)	0.83	0.93	1.1	—	[62]
		Hexane (100)	2.4	2.9	1.2	—	
cis-Chlordane	Chiralcel-OD	Hexane/isopropanol (99–1)	0.87	1.0	1.2	—	
		Hexane (100)	1.7	3.2	1.9	—	
Heptachlor	Chiralcel-OD	Hexane (100)	0.80	0.97	1.2	—	
α-HCH	Chiralcel-OJ	Hexane/isopropanol (90-10)	1.0	1.4	1.4	—	
Heptachlor epoxide	Chiralpak-AD	MeOH	0.37	0.64	1.7	—	
Fipronil	Cellulose-tri (3,5-DMPC)	Hexane/ethanol (80–20)	0.78	—	1.00	0.0	[63]
		Hexane/ethanol (85–15)	0.99	—	1.12	0.25	
		Hexane/ethanol (90-10)	1.69	—	1.14	0.63	
		Hexane/ethanol (95–05)	4.27	—	1.18	1.01	
		Hexane/ethanol (98–02)	10.70	—	1.22	1.27	
		Hexane/*n*-propanol (80–20)	0.90	—	1.00	0.0	
		Hexane/*n*-propanol (85–15)	1.40	—	1.00	0.0	
		Hexane/*n*-propanol (90–10)	2.18	—	1.13	0.46	
		Hexane/*n*-propanol (95–05)	4.94	—	1.19	1.06	
		Hexane/*n*-propanol (98–02)	11.83	—	1.21	1.27	
		Hexane/isopropanol (80–20)	0.90	—	1.26	0.0	
		Hexane/isopropanol (85–15)	1.38	—	1.28	0.46	
		Hexane/isopropanol (90–10)	2.54	—	1.31	0.91	
		Hexane/isopropanol (95–05)	6.06	—	1.36	1.37	

		Hexane/isopropanol (98–02)	15.56	—	1.37	1.69
		Hexane/*n*-butanol (80–20)	1.47	—	1.00	0.0
		Hexane/*n*-butanol (85–15)	1.38	—	1.00	0.0
		Hexane/*n*-butanol (90–10)	2.14	—	1.12	0.38
		Hexane/*n*-butanol (95–150)	4.85	—	1.17	1.05
		Hexane/*n*-butanol (98–02)	11.28	—	1.20	1.22
		Hexane/isobutanol (80–20)	1.16	—	1.29	0.0
		Hexane/isobutanol (85–15)	1.83	—	1.35	1.26
		Hexane/isobutanol (90–10)	3.15	—	1.37	1.47
		Hexane/isobutanol (95–05)	7.42	—	1.38	1.81
		Hexane/isobutanol (98–02)	—	—	—	—
Isocarbophos	Cellulose-tris (3,5-DMPC)	Hexane/ethanol (80–20)	0.77	—	1.17	0.30
		Hexane/ethanol (85–15)	0.97	—	1.23	0.51
		Hexane/ethanol (90–10)	1.21	—	1.34	0.78
		Hexane/ethanol (95–05)	2.13	—	1.25	1.32
		Hexane/ethanol (98–02)	3.69	—	1.27	1.61
		Hexane/*n*-propanol (80–20)	0.87	—	1.34	0.96
		Hexane/*n*-propanol (85–15)	1.05	—	1.33	1.14
		Hexane/*n*-propanol (90–10)	1.56	—	1.37	1.40
		Hexane/*n*-propanol (95–05)	2.61	—	1.40	1.81
		Hexane/*n*-propanol (98–02)	4.49	—	1.41	2.63
		Hexane/isopropanol (80–20)	0.96	—	1.52	1.47
		Hexane/isopropanol (85–15)	1.43	—	1.60	1.79
		Hexane/isopropanol (90–10)	1.90	—	1.67	2.18

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Hexane/isopropanol (95–05)	3.26	—	1.89	2.66	
		Hexane/isopropanol (98–02)	5.93	—	1.66	2.42	
		Hexane/*n*-butanol (80–20)	0.94	—	1.28	0.87	
		Hexane/*n*-butanol (85–15)	1.09	—	1.28	0.91	
		Hexane/*n*-butanol (90–10)	1.50	—	1.30	1.27	
		Hexane/*n*-butanol (95–05)	2.42	—	1.32	1.38	
		Hexane/*n*-butanol (98–02)	4.90	—	1.39	1.86	
		Hexane/isobutanol (80–20)	1.01	—	1.40	1.23	
		Hexane/isobutanol (85–15)	1.32	—	1.43	1.56	
		Hexane/isobutanol (90–10	1.65	—	1.43	1.61	
		Hexane/isobutanol (95–05)	1.34	—	2.70	1.65	
		Hexane/isobutanol (98–02)	6.27	—	1.68	2.56	
Carfentrazone-ethyl	Cellulose-tri (3,5-DMPC)	Hexane/isopropanol (80–20)	0.78	—	1.00	0.0	
		Hexane/isopropanol (85–15)	0.91	—	1.00	0.0	
		Hexane/isopropanol (90–10)	1.08	—	1.10	0.24	
		Hexane/isopropanol (95–05)	1.47	—	1.10	0.33	
		Hexane/isopropanol (98–02)	2.49	—	1.10	0.45	
		Hexane/isopropanol (99–01)	11.72	—	1.08	0.52	
		Hexane/isopropanol (99.5–0.5)	5.45	—	1.07	0.58	
		Hexane/isopropanol (99.9–0.1)	3.53	—	1.09	0.48	
λ-cyhalothrin	Chiralpak AD	Hexane/ethanol (95–05)	0.56	0.67	1.20	1.60	[67]
		Hexane/ethanol (96–04)	0.60	0.72	1.24	1.81	

		Hexane/ethanol (97–03)	0.63	0.81	1.28	2.06	
		Hexane/ethanol (99–01)	0.84	1.10	1.30	2.20	
		Hexane/ethanol (98–02)	1.10	1.35	1.32	2.35	
	Chiralpak AS	Hexane/1, 2-dichloroethane (95–05)	1.05	1.94	1.85	4.96	
		Hexane/1, 2-dichloroethane (96–04)	1.72	3.19	1.86	4.90	
		Hexane/1, 2-dichloroethane (97–03)	2.98	5.65	1.90	4.54	
		Hexane/1, 2-dichloroethane (98–02)	4.56	8.44	1.85	4.18	
		Hexane/1, 2-dichloroethane (99–01)	6.70	2.19	1.82	3.17	
	Chiralcel OD	Hexane/isopropanol (95–05)	1.18	1.75	1.49	5.95	
		Hexane/isopropanol (96–04)	1.25	1.92	1.53	6.54	
		Hexane/isopropanol (97–03)	1.79	2.34	1.55	7.31	
		Hexane/isopropanol (98–02)	2.24	3.59	1.60	8.41	
		Hexane/isopropanol (99–01)	3.33	5.76	1.73	10.41	
	Chiralcel OJ	Hexane/ethanol (95–05)	1.56	1.99	1.27	3.22	
		Hexane/ethanol (96–04)	1.82	2.34	1.28	3.50	
		Hexane/ethanol (97–03)	2.24	2.90	1.30	3.82	
		Hexane/ethanol (98–02)	2.92	3.87	1.33	4.30	
		Hexane/ethanol (99–01)	4.52	6.13	1.36	4.79	
Salithion	Chiralcel OJ	Hexane/isopropanol (95–05)	8.47	9.32	1.10	1.56	[70]
	Chiralcel OD	Hexane/isopropanol (99.5–0.5)	6.76	7.40	1.09	1.42	
	Chiralpak AD	Hexane/isopropanol (99.5–0.5)	4.15	5.03	1.21	4.14	
	Chiralpak OT (+)	Methanol (100)	2.02	2.52	1.25	2.28	
Neonicotinoid 1	Chiralcel OD-H	Hexane/ethanol (40–60)	1.40	1.97	1.41	2.74	[73]
		Hexane/ethanol (50–50)	1.92	2.65	1.38	2.75	
		Hexane/ethanol (60–40)	2.98	4.05	1.36	2.77	

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Hexane/ethanol (70–30)	5.25	7.01	1.34	2.87	
		Hexane/ethanol (80–20)	11.75	15.46	1.32	3.15	
Neonicotinoid 2	Chiralcel OD-H	Hexane/ethanol (40–60)	0.69	1.13	1.62	1.98	
		Hexane/ethanol (50–50)	1.02	1.67	1.64	2.15	
		Hexane/ethanol (60–40)	1.70	2.82	1.67	2.42	
		Hexane/ethanol (70–30)	3.26	5.57	1.71	2.84	
		Hexane/ethanol (80–20)	7.79	13.69	1.76	3.43	
Neonicotinoid 3	Chiralcel OD-H	Hexane/ethanol (50–50)	0.41	0.49	1.18	0.98	
		Hexane/ethanol (60–40)	0.64	0.75	1.17	1.09	
		Hexane/ethanol (70–30)	1.18	1.39	1.17	1.19	
		Hexane/ethanol (80–20)	2.50	2.90	1.16	1.27	
		Hexane/ethanol (90–10)	8.54	9.88	1.57	1.49	
Metalaxyl	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	3.01	4.66	1.55	2.51	[76]
		Hexane/isopropanol (90–10)	4.28	6.68	1.56	2.80	
		Hexane/isopropanol (95–05)	7.56	12.27	1.62	3.75	
Myclobutanil	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	5.08	5.96	1.17	0.86	
		Hexane/isopropanol (90–10)	8.01	9.59	1.20	1.06	
		Hexane/isopropanol (95–05)	17.49	21.74	1.24	1.49	
Imazalil	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	2.50	2.70	1.08	0.52	
		Hexane/isopropanol (90–10)	3.71	4.08	1.10	0.70	
		Hexane/isopropanol (95–05)	7.56	8.44	1.12	0.86	
Malathion	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (90–10)	2.49	2.49	1.00	0.0	

		Hexane/isopropanol (98–02)	3.19	3.48	1.09	0.77	
		Hexane/isopropanol (95–05)	4.53	5.02	1.11	0.87	
Triadimefon	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	1.81	2.33	1.29	1.34	
		Hexane/isopropanol (90–10)	2.48	3.27	1.32	1.43	
		Hexane/isopropanol (95–05)	3.74	5.07	1.35	1.59	
		Hexane/isopropanol (98–02)	5.97	8.47	1.42	1.84	
Ethofumesate	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (90–10)	4.71	4.71	1.00	0.0	
		Hexane/isopropanol (95–05)	6.66	6.92	1.04	0.40	
		Hexane/isopropanol (98–02)	9.95	10.58	1.06	0.63	
Fipronil	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	2.83	3.45	1.22	1.03	
		Hexane/isopropanol (90–10)	7.72	9.88	1.28	1.35	
		Hexane/isopropanol (95–05)	14.63	19.42	1.33	2.03	
Napropamide	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	1.55	1.66	1.07	0.38	
		Hexane/isopropanol (90–10)	2.04	2.25	1.10	0.55	
		Hexane/isopropanol (95–05)	3.31	3.66	1.11	0.72	
		Hexane/isopropanol (98–02)	7.65	8.76	1.14	1.14	
Paclobutrazol	Amylose tris-(*S*)-1-(PEC)	Hexane/isopropanol (85–15)	2.03	2.36	1.16	0.81	
		Hexane/isopropanol (90–10)	3.57	4.29	1.20	1.30	
		Hexane/isopropanol (95–05)	6.38	7.74	1.21	1.66	
Metalaxyl	Amylopectin-tris-(PC)	Hexane/isopropanol (80–20)	4.57	5.34	1.17	0.94	[79]
		Hexane/isopropanol (85–15)	5.74	6.69	1.16	0.97	
		Hexane/isopropanol (90–10)	9.11	10.77	1.18	1.37	
Hexaconazole	Amylopectin-tris-(PC)	Hexane/isopropanol (80–20)	1.78	2.38	1.34	1.49	
		Hexane/isopropanol (85–15)	2.33	3.19	1.36	1.83	
		Hexane/isopropanol (90–10)	3.60	4.99	1.39	2.03	

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Hexane/isopropanol (95–05)	7.53	10.60	1.41	2.45	
Myclobutanil	Amylopectin-tris-(PC)	Hexane/isopropanol (70–30)	3.72	4.55	1.22	0.86	
		Hexane/isopropanol (80–20)	5.63	6.93	1.23	1.10	
		Hexane/isopropanol (85–15)	8.17	10.04	1.23	1.20	
		Hexane/isopropanol (90–10)	13.11	16.27	1.24	1.47	
Tebuconazole	Amylopectin-tris-(PC)	Hexane/isopropanol (80–20)	2.07	2.38	1.15	0.63	
		Hexane/isopropanol (85–15)	2.80	3.32	1.19	0.92	
		Hexane/isopropanol (90–10)	4.37	5.39	1.23	1.34	
		Hexane/isopropanol (95–05)	9.84	12.01	1.22	1.54	
Uniconazole	Amylopectin-tris-(PC)	Hexane/isopropanol (80–20)	2.11	2.13	1.01	0.05	
		Hexane/isopropanol (85–15)	2.31	3.02	1.31	1.39	
		Hexane/isopropanol (90–10)	3.86	5.17	1.34	1.48	
		Hexane/isopropanol (95–05)	9.33	13.09	1.40	2.05	
Paclobutrazol	Amylopectin-tris-(PC)	Hexane/isopropanol (85–15)	2.30	3.41	1.48	1.61	
		Hexane/isopropanol (90–10)	3.79	5.88	1.55	2.19	
		Hexane/isopropanol (95–05)	8.16	12.65	1.55	2.42	
Benalaxyl	Amylopectin-tris-(PC)	Hexane/isopropanol (85–15)	2.59	2.91	1.12	0.66	
		Hexane/isopropanol (90–10)	3.63	4.12	1.13	0.77	
		Hexane/isopropanol (95–05)	4.75	5.59	1.18	0.83	
		Hexane/isopropanol (97–03)	8.79	10.32	1.17	1.01	
EPN	Chiralpak AD	Hexane/isopropanol (90–10)	0.86	0.96	1.11	1.13	[80]
		Hexane/isopropanol (95–05)	0.90	1.04	1.16	1.41	

		Hexane/isopropanol (96–04)	0.89	1.05	1.18	1.61	
		Hexane/isopropanol (97–03)	0.94	1.08	1.15	2.31	
		Hexane/isopropanol (98–02)	1.17	1.46	1.25	3.22	
		Hexane/isopropanol (99–01)	1.86	2.61	1.40	5.39	
	Chiralpak AS	Hexane/isopropanol (90–10)	1.23	1.45	1.18	1.89	
		Hexane/isopropanol (95–05)	1.33	1.59	1.19	2.11	
		Hexane/isopropanol (96–04)	1.56	1.87	1.19	2.28	
		Hexane/isopropanol (97–03)	1.48	1.70	1.15	1.87	
		Hexane/isopropanol (98–02)	1.93	2.24	1.16	2.13	
		Hexane/isopropanol (99–01)	2.27	2.68	1.18	2.50	
o,p-DDT	Chiralpak AD-RH	Acetonitrile/water (50–50)	15.41	19.77	1.24	2.47	[59]
o,p-DDD		Acetonitrile/water (50–50)	—	—	—	—	
o,p-DDT	Chiralpak AD-RH	Acetonitrile/isopropanol (50–50)	4.74	8.00	1.69	1.00	
o,p-DDD		Acetonitrile/isopropanol (50–50)	3.26	4.11	1.26	0.60	
o,p-DDT	Chiralcel OD-RH	Acetonitrile/water (50–50)	4.54	10.28	2.27	2.03	
o,p-DDD		Acetonitrile/water (50-50)	—	—	—	—	
o,p-DDT	Chiralcel OJ-R	Acetonitrile/isopropanol (50–50)	—	—	—	—	
o,p-DDD		Acetonitrile/isopropanol (50–50)	—	—	—	—	
o,p-DDT		Acetonitrile/water (50–50)	3.49	8.80	2.52	0.80	
o,p-DDD		Acetonitrile/water (50–50)	—	—	—	—	
o,p-DDT		Acetonitrile/isopropanol (50–50)	—	—	—	—	
o,p-DDD		Acetonitrile/isopropanol (50–50)	—	—	—	—	
Epoxiconazole	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	4.38	8.26	1.88	5.54	[81]
		Methanol/water (80–20)	2.79	5.40	1.93	5.45	
		Methanol/water (85–15)	1.74	3.34	1.92	5.32	

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Methanol/water (90–10)	1.16	2.22	1.91	5.05	
		Methanol/water (95–5)	0.90	1.75	1.96	5.00	
		Methanol/water (100–0)	1.01	1.62	1.60	3.41	
		Acetonitrile/water (50–50)	4.18	8.39	2.00	9.23	
		Acetonitrile/water (60–40)	1.85	3.86	2.08	7.71	
		Acetonitrile/water (70–30)	1.03	2.16	2.09	6.08	
		Acetonitrile/water (80–20)	0.65	1.39	2.12	5.14	
		Acetonitrile/water(90–10)	0.56	1.09	1.96	4.21	
		Acetonitrile/water (100–0)	0.95	1.75	1.84	4.83	
Terallethrin	Cellulose-tris-(3,5-DMPC)	Methanol/water (65–35)	15.09	15.91	1.05	0.66	
		Methanol/water (70–30)	8.18	8.58	1.05	0.54	
		Methanol/water (75–25)	4.52	4.74	1.05	0.53	
		Methanol/water (80–20)	2.63	2.74	1.04	0.42	
		Methanol/water (90–10)	1.01	1.01	1.00	—	
		Methanol/water (100–0)	0.70	0.70	1.00	—	
		Acetonitrile/water (50–50)	5.66	6.28	1.11	1.56	
		Acetonitrile/water (60–40)	2.18	2.43	1.12	1.22	
		Acetonitrile/water (70–30)	1.03	1.15	1.12	0.89	
		Acetonitrile/water (80–20)	0.53	0.60	1.13	0.66	
		Acetonitrile/water (90–10)	0.33	0.33	1.00	—	
		Acetonitrile/water (100–0)	0.51	0.51	1.00	—	
Pyriproxyfen	Cellulose-tris-(3,5-DMPC)	Methanol/water (80–20)	12.01	13.05	1.09	0.93	

		Methanol/water (85–15)	5.99	6.43	1.07	0.66
		Methanol/water (90–10)	3.20	3.38	1.06	0.61
		Methanol/water (95–05)	2.04	2.04	1.00	—
		Methanol/water (100–0)	1.13	1.13	1.00	—
		Acetonitrile/water (55–45)	12.38	13.09	1.06	0.91
		Acetonitrile/water (60–40)	7.38	7.82	1.06	0.84
		Acetonitrile/water (70–30)	3.20	3.39	1.06	0.72
		Acetonitrile/water (80–20)	1.52	1.58	1.04	0.47
		Acetonitrile/water (90–10)	0.75	0.75	1.00	—
		Acetonitrile/water (100–0)	0.45	0.45	1.00	—
Benalaxyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	4.02	4.82	1.20	1.59
		Methanol/water (85–15)	1.54	1.83	1.19	1.35
		Methanol/water (90–10)	1.04	1.22	1.17	1.13
		Methanol/water (95–05)	0.76	0.88	1.16	0.92
		Methanol/water (100–0)	0.64	0.72	1.14	0.76
		Acetonitrile/water (40–60)	14.20	14.20	1.00	—
		Acetonitrile/water (80–20)	0.54	0.54	1.00	—
		Acetonitrile/water (100–0)	0.38	0.38	1.00	—
Lactofen	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	14.71	6.26	1.11	1.07
		Methanol/water (80–20)	7.01	7.75	1.10	0.92
		Methanol/water (85–15)	3.13	3.46	1.11	0.83
		Methanol/water (90–10)	1.52	1.69	1.11	0.66
		Methanol/water (95–05)	0.79	0.88	1.11	0.63
		Methanol/water (100–0)	0.77	0.77	1.00	—
		Acetonitrile/water (50–50)	15.19	15.19	1.00	—

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Acetonitrile/water (80–20)	0.57	0.57	1.00	—	
		Acetonitrile/water (100–0)	0.44	0.44	1.00	—	
Quizalofop-ethyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	21.48	22.61	1.05	0.69	
		Methanol/water (80–20)	11.40	12.02	1.05	0.59	
		Methanol/water (85–15)	6.06	6.38	1.05	0.54	
		Methanol/water (90–10)	3.40	3.56	1.05	0.45	
		Methanol/water (95–05)	2.11	2.11	1.00	—	
		Methanol/water (100-0)	1.20	1.20	1.00	—	
		Acetonitrile/water (50–50)	13.04	13.04	1.00	—	
		Acetonitrile/water (80–20)	1.02	1.02	1.00	—	
		Acetonitrile/water (100–0)	0.45	0.45	1.00	—	
Diclofop-methyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	14.43	14.43	1.00	—	
		Methanol/water (85–15)	4.43	4.43	1.00	—	
		Methanol/water (100–0)	0.77	0.77	1.00	—	
		Acetonitrile/water (50–50)	12.73	13.95	1.10	1.53	
		Acetonitrile/water (60–40)	4.35	4.76	1.09	1.22	
		Acetonitrile/water (70–30)	1.87	2.05	1.10	0.97	
		Acetonitrile/water (80–20)	0.87	0.95	1.10	0.72	
		Acetonitrile/water (90–10)	0.86	0.86	1.00	—	
		Acetonitrile/water (100–0)	0.34	0.34	1.00	—	
Profenofos	Cellulose-tris-(3,5-DMPC)	Methanol/water (70–30)	12.90	12.90	1.00	—	
		Methanol/water (80–20)	4.25	4.25	1.00	—	

		Methanol/water (100–0)	0.93	0.93	1.00	—
		Acetonitrile/water (50–50)	8.47	8.47	1.00	—
		Acetonitrile/water (70–30)	1.83	1.83	1.00	—
		Acetonitrile/water (100–0)	0.62	0.62	1.00	—
Malathion	Cellulose-tris-(3,5-DMPC)	Methanol/water (70–30)	6.90	6.90	1.00	—
		Methanol/water (80–20)	2.41	2.41	1.00	—
		Methanol/water (100–0)	0.77	0.77	1.00	—
		Acetonitrile/water (70–30)	0.85	0.85	1.00	—
		Acetonitrile/water (100–0)	0.49	0.49	1.00	—
Methamidophos	Cellulose-tris-(3,5-DMPC)	Methanol/water (60–40)	1.28	1.28	1.00	—
		Methanol/water (80–20)	1.82	1.82	1.00	—
		Methanol/water (100–0)	0.76	0.76	1.00	—
		Acetonitrile/water (40–60)	0.97	0.97	1.00	—
		Acetonitrile/water (70–30)	2.35	2.35	1.00	—
		Acetonitrile/water (100–0)	0.96	0.96	1.00	—
MCPA-isooctyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (80–20)	12.57	12.57	1.00	—
		Methanol/water (90–10)	2.88	2.88	1.00	—
		Methanol/water (100–0)	1.12	1.12	1.00	—
		Acetonitrile/water (60-40)	10.37	10.37	1.00	—
		Acetonitrile/water (80–20)	1.88	1.88	1.00	—
		Acetonitrile/water (100–0)	0.79	0.79	1.00	—
Isofenphos-methyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (70–30)	5.49	5.49	1.00	—
		Methanol/water (80–20)	1.99	1.99	1.00	—
		Methanol/water (100–0)	0.65	0.65	1.00	—
		Acetonitrile/water (50–50)	5.21	5.21	1.00	—

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Acetonitrile/water (70–30)	1.07	1.07	1.00	—	
		Acetonitrile/water (100–0)	0.53	0.53	1.00	—	
Phenthoate	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	7.54	7.54	1.00	—	
		Methanol/water (90–10)	1.39	1.39	1.00	—	
		Methanol/water (100–0)	0.87	0.87	1.00	—	
		Acetonitrile/water (50–50)	6.57	6.57	1.00	—	
		Acetonitrile/water (70–30)	1.26	1.26	1.00	—	
		Acetonitrile/water (100–0)	0.57	0.57	1.00	—	
Fluroxypyr-meptyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (75–25)	14.85	14.85	1.00	—	
		Methanol/water (90–10)	1.94	1.94	1.00	—	
		Methanol/water (100–0)	0.80	0.80	1.00	—	
		Acetonitrile/water (50–50)	17.75	17.75	1.00	—	
		Acetonitrile/water (70–30)	2.33	2.33	1.00	—	
		Acetonitrile/water (100–0)	0.65	0.65	1.00	—	
Acephate	Cellulose-tris-(3,5-DMPC)	Methanol/water (60–40)	0.97	0.97	1.00	—	
		Methanol/water (80–20)	0.88	0.88	1.00	—	
		Methanol/water (100–0)	0.76	0.76	1.00	—	
		Acetonitrile/water (50–50)	0.76	0.76	1.00	—	
		Acetonitrile/water (80–20)	0.84	0.84	1.00	—	
		Acetonitrile/water (100–0)	0.93	0.93	1.00	—	
Trichlorphon	Cellulose-tris-(3,5-DMPC)	Methanol/water (60–40)	1.33	1.33	1.00	—	
		Methanol/water (80–20)	0.87	0.87	1.00	—	

		Methanol/water (100–0)	1.07	1.07	1.00	—
		Acetonitrile/water (80–20)	0.61	0.61	1.00	—
		Acetonitrile/water (100–0)	0.99	0.99	1.00	—
2,4-D-ethylhexyl	Cellulose-tris-(3,5-DMPC)	Methanol/water (80–20)	13.38	13.38	1.00	—
		Methanol/water (90–10)	3.13	3.13	1.00	—
		Methanol/water (100–0)	1.17	1.17	1.00	—
		Acetonitrile/water (60–40)	10.06	10.06	1.00	—
		Acetonitrile/water (80–20)	1.84	1.84	1.00	—
		Acetonitrile/water (100–0)	0.78	0.78	1.00	—
Fenamiphos	Cellulose-tris-(3,5-DMPC)	Methanol/water (70–30)	3.17	3.17	1.00	—
		Methanol/water (90–10)	0.85	0.85	1.00	—
		Methanol/water (100–0)	0.62	0.62	1.00	—
		Acetonitrile/water (40–60)	6.45	6.45	1.00	—
		Acetonitrile/water (70–30)	0.81	0.81	1.00	—
		Acetonitrile/water (100–0)	1.69	1.69	1.00	—
Acetochlor	Cellulose-tris-(3,5-DMPC)	Methanol/water (70–30)	4.33	4.33	1.00	—
		Methanol/water (90–10)	1.25	1.25	1.00	—
		Methanol/water (100–0)	0.82	0.82	1.00	—
		Acetonitrile/water (50–50)	4.22	4.22	1.00	—
		Acetonitrile/water (70–30)	1.13	1.13	1.00	—
		Acetonitrile/water (100–0)	0.66	0.66	1.00	—
P-tefuryl quizalofop-	Cellulose-tris-(3,5-DMPC)	Methanol/water (85–15)	9.46	9.46	1.00	—
		Methanol/water (90–10)	1.75	1.75	1.00	—
		Methanol/water (100–0)	5.59	5.59	1.00	—
		Acetonitrile/water (60–40)	5.55	5.55	1.00	—

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Acetonitrile/water (70–30)	0.98	0.98	1.00	—	
		Acetonitrile/water (100–0)	2.76	2.76	1.00	—	
Hexaconazole	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	3.20	—	1.15	3.94	[82]
		Acetonitrile/water (70–30)	0.87	—	1.17	2.98	
		Acetonitrile/water (90–10)	0.44	—	1.19	2.25	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	3.41	—	1.16	3.09	
		Acetonitrile/water (70–30)	0.88	—	1.17	1.71	
		Acetonitrile/water (90–10)	0.48	—	1.17	1.26	
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	5.45	—	1.14	2.59	
		Methanol/water (80–20)	1.75	—	1.13	2.12	
		Methanol/water (90–10)	0.66	—	1.13	1.44	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	4.86	—	1.12	1.50	
		Methanol/water (80–20)	1.71	—	1.09	0.76	
		Methanol/water (90–10)	—	—	—	—	
Flutriafol	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	1.45	—	1.12	2.55	
		Acetonitrile/water (70–30)	0.45	—	1.14	1.99	
		Acetonitrile/water (90–10)	0.27	—	1.16	1.35	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	1.52	—	1.12	1.44	
		Acetonitrile/water (70–30)	0.49	—	1.07	0.50	
		Acetonitrile/water (90–10)	—	—	—	—	
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	2.13	—	1.08	1.39	
		Methanol/water (80–20)	0.87	—	1.08	1.06	

		Methanol/water (90–10)	—	—	—	—
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	—	—	—	—
		Methanol/water (80–20)	—	—	—	—
		Methanol/water (90–10)	—	—	—	—
Diniconazole	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	3.71	—	1.11	3.09
		Acetonitrile/water (70–30)	0.92	—	1.12	2.31
		Acetonitrile/Water (90–10)	0.4	—	1.11	1.22
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	3.98	—	1.34	6.71
		Acetonitrile/water (70–30)	0.93	—	1.13	1.30
		Acetonitrile/water (90–10)	—	—	—	—
Tetraconazole	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	4.15	—	1.29	7.37
		Acetonitrile/water (70–30)	0.86	—	1.31	5.04
		Acetonitrile/water (90–10)	0.30	—	1.39	3.35
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	4.49	—	1.29	5.79
		Acetonitrile/water (70–30)	0.87	—	1.30	2.85
		Acetonitrile/water (90–10)	0.34	—	1.33	1.66
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	—	—	—	—
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	—	—	—	—
Epoxiconazole	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	4.22	—	2.04	20.90
		Acetonitrile/water (70–30)	1.03	—	2.10	16.89
		Acetonitrile/water (90–10)	0.42	—	2.27	12.3
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	4.57	—	2.00	16.71
		Acetonitrile/water (70–30)	1.06	—	2.03	10.45
		Acetonitrile/water (90–10)	0.47	—	2.08	7.06
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	8.71	—	1.29	5.86

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
		Methanol/water (80–20)	2.93	—	1.62	9.56	
		Methanol/water (90–10)	1.16	—	1.75	9.42	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	7.72	—	1.49	6.51	
		Methanol/water (80–20)	2.78	—	1.55	5.90	
		Methanol/water (90–10)	1.19	—	1.67	5.19	
Penconazole	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	4.20	—	1.22	7.58	
		Acetonitrile/water (70–30)	1.34	—	1.05	1.18	
		Acetonitrile/water (90–10)	0.61	—	1.06	1.00	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	5.29	—	1.05	1.12	
		Acetonitrile/water (70–30)	—	—	—	—	
		Acetonitrile/water (90–10)	—	—	—	—	
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	6.57	—	1.18	3.87	
		Methanol/water (80–20)	2.31	—	1.17	3.20	
		Methanol/water (90–10)	0.95	—	1.17	2.29	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	5.95	—	1.15	2.54	
		Methanol/water (80–20)	2.23	—	1.15	1.68	
		Methanol/water (90–10)	0.98	—	1.15	1.15	
Myclobutanil	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	3.44	—	1.42	9.99	
		Acetonitrile/water (70–30)	0.88	—	1.44	7.58	
		Acetonitrile/water (90–10)	0.38	—	1.51	5.10	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	3.69	—	1.42	7.72	
		Acetonitrile/water (70–30)	0.9	—	1.44	4.28	

		Acetonitrile/water (90–10)	0.42	—	1.45	2.85
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	5.07	—	1.26	4.47
		Methanol/water (80–20)	1.91	—	1.31	4.75
		Methanol/water (90–10)	0.87	—	1.41	4.91
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	4.53	—	1.24	3.06
		Methanol/water (80–20)	1.83	—	1.27	2.43
		Methanol/water (90–10)	0.89	—	1.37	2.25
Fenbuconazole	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	6.86	—	1.33	9.03
		Acetonitrile/water (70–30)	1.48	—	1.34	7.38
		Acetonitrile/water (90–10)	0.56	—	1.37	4.79
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	7.42	—	1.33	7.48
		Acetonitrile/water (70–30)	1.49	—	1.34	4.46
		Acetonitrile/water (90–10)	0.59	—	1.34	2.80
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	16.1	—	1.21	3.96
		Methanol/water (80–20)	5.09	—	1.24	4.35
		Methanol/water (90–10)	1.97	—	1.29	4.80
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	13.1	—	1.21	3.23
		Methanol/water (80–20)	4.76	—	1.23	3.17
		Methanol/water (90–10)	1.96	—	1.28	2.89
Triadimefon	Cellulose-tris-(3,5-DMPC) (5 μM)	Acetonitrile/water (50–50)	2.72	—	1.15	3.82
		Acetonitrile/water (70–30)	0.63	—	1.17	2.43
		Acetonitrile/water (90–10)	0.22	—	1.20	1.45
	Cellulose-tris-(3,5-DMPC) (3 μM)	Acetonitrile/water (50–50)	2.87	—	1.16	2.67
		Acetonitrile/water (70–30)	0.65	—	1.17	1.26
		Acetonitrile/water (90–10)	—	—	—	—

(continued)

Table 2.2 (*Continued*)

Pesticides	Column/CSPs	Mobile phase (v/v)	k_1	k_2	α	R_s	References
	Cellulose-tris-(3,5-DMPC) (5 μM)	Methanol/water (70–30)	3.70	—	1.29	5.15	
		Methanol/water (80–20)	1.28	—	1.29	4.13	
		Methanol/water (90–10)	0.51	—	1.28	2.73	
	Cellulose-tris-(3,5-DMPC) (3 μM)	Methanol/water (70–30)	3.22	—	1.27	2.66	
		Methanol/water (80–20)	1.26	—	1.26	1.59	
		Methanol/water (90–10)	0.55	—	1.23	1.00	

DMPC: dimethylphenylcarbamate; PEC: phenylethylcarbamate; PC: phenylcarbamate.

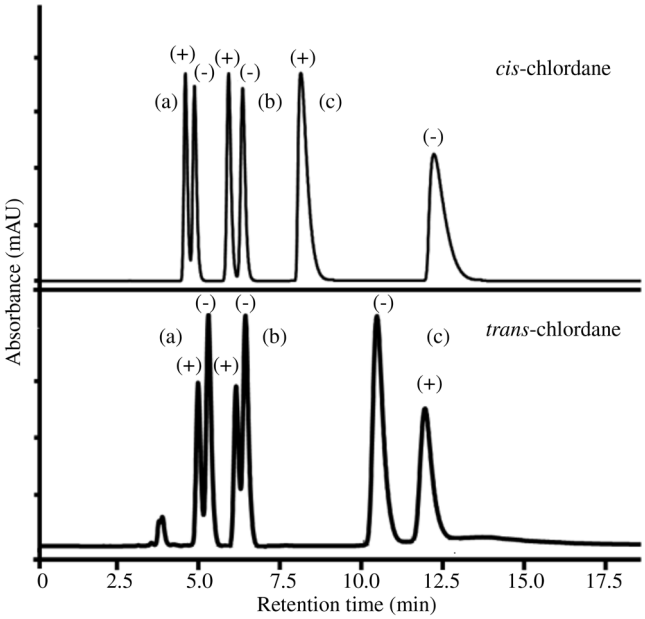


Figure 2.3 Effect of the concentration of isopropanol (IPA) on chiral resolution of *cis*-chlordane and *trans*-chlordane on Chiralcel OD column. (a) Hexane–isopropanol (97:3, v/v), (b) hexane–isopropanol (99:1, v/v), and (c) hexane (100%) [62].

2-dichloroethane (500:1, v/v) and hexane-1,2-dichloroethane-ethanol (500:10:0.05, v/v/v), respectively. Lin *et al.* [66] performed the chiral separation of methamidophos on Chiralcel OD column with the mobile phase of *n*-hexane-isopropanol (80:20, v/v) at the 0.5 ml/min flow rate on OR and CD detectors and confirmed that *R*-(+)-methamidophos eluted before *S*-(−)-methamidophos at 230 nm. Similarly, the same group of researchers also studied the chiral separation of fosthiazate that possesses two stereogenic centers on Chiralpak AD column with a mobile phase consisting of *n*-hexane-ethanol (95:5, v/v) and the 1.0 ml/min flow rate. The identification of peaks has been confirmed with OR and CD detectors at 230 nm and the values of capacity, separation, and resolution factors varies with a change of concentration of ethanol as shown in Table 2.3 [67]. Furthermore, Xu *et al.* [68] carried out the enantiomeric resolution of pyrethroid insecticides (λ-cyhalothrin) with Chiralpak AD (amylase tris [3,5-dimethylphenyl carbamate]), Chiralpak AS (amylose tris[(*S*)-1-phenyl carbamate]), Chiralcel OD (cellulose tris[3,5-dimethylphenyl carbamate]), and Chiralcel OJ (cellulose tris[4-methyl benzoate]) chiral stationary phases. In this study, they concluded that all the chiral stationary phases are good for the resolution when different ratios of eluting solvents are used as shown in Table 2.2 and confirmed that 5% ethanol is a good modifier in all the cases examined. They also studied the effect of temperature on the chiral resolution with two different

Table 2.3 Effect of concentration of ethanol on the chiral resolution of *R/S* methamidophos [65,73].

Mobile phase (hexane-ethanol)	Capacity factor (k)				Separation factor (α)						Resolution (R_s)		
	k_1	k_2	k_3	k_4	α_{12}	α_{23}	α_{34}	α_{14}	α_{13}	α_{24}	R_{s12}	R_{s23}	R_{s34}
(95:05, v/v)	2.69	3.10	8.60	13.82	1.15	2.77	1.61	5.13	3.19	4.45	1.56	14.85	9.21
(90:10, v/v)	1.37	1.55	4.16	6.73	1.13	2.68	1.62	4.92	3.04	4.33	1.12	12.24	8.05
(85:15, v/v)	0.90	1.01	2.62	4.25	1.13	2.59	1.62	4.73	2.91	4.2	0.92	9.99	7.58

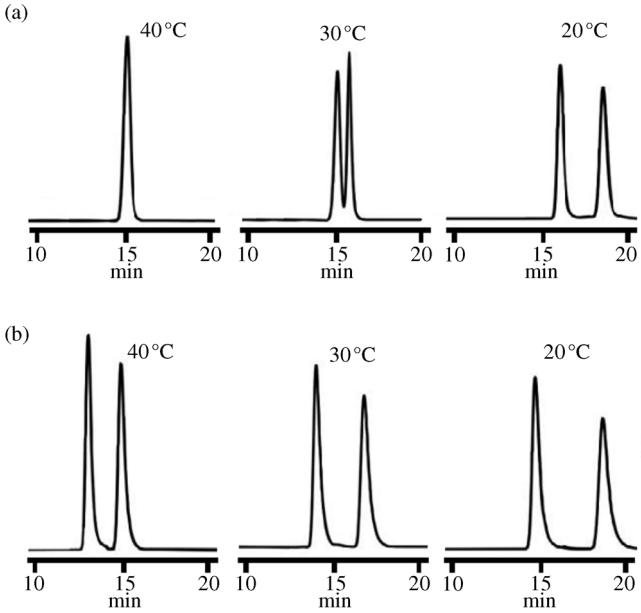
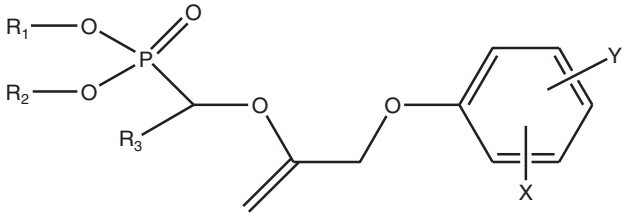


Figure 2.4 Effect of temperature on chiral separation of λ-cyhalothrin with two chiral stationary phases. (a) Chiralpak amylose tris-(3,5-dimethylphenyl-carbamate), *n*-hexane-ethanol (99:1, v/v), 0.40 ml/min; (b) Chiralpak amylose tris-([*S*]-α-methylbenzyl-carbamate), *n*-hexane-ethanol (97.5:2.5, v/v), 0.040 ml/min [67].

columns as shown in Figure 2.4 and confirmed that 20 °C is the optimum temperature for the baseline separation. Li *et al.* [69] developed methods for the chiral resolution of five organophosphorus compounds (Figure 2.5) on Chiralpak AD, Chiralpak AS, Chiralcel OD, and Chiralcel OJ columns. The baseline separations of all these compounds were obtained on Chiralpak AD column by using different ratios of hexane-ethanol and hexane-isopropanol as the mobile phase. Compound 1 was separated with hexane-ethanol (90:10, v/v), while compounds 2, 3, 4, and 5 had eluted with hexane-isopropanol (90 : 10, v/v) and (95: 5, v/v). Zhou *et al.* [70] also studied the enantiomeric resolution of salithion on different chiral stationary phases (Chiralcel OD, Chiralcel OJ, and Chiralpak AD) with different concentrations of isopropanol with hexane at the 1.0 ml/min flow rate, and the best separation was observed on Chiralpak AD column with hexane-isopropanol (99.5/0.5, v/v), and various chromatographic values measured are shown in Table 2.2. The same group of scholars resolved a long series of triazole fungicides (hexaconazole, triadimefon, tebuconazole, diniconazole, flutriafol, propiconazole, and difenoconazole) on Chiralcel OD and Chiralcel OJ columns. These authors reported that four compounds, namely, hexaconazole, triadimefon, tebuconazole, and diniconazole had been separated on Chiralcel OD column. The enantiomers of flutriafol had been obtained by changing the mobile



Organophosphorous Compound	R1	R2	R3	X	Y
1	C_2H_5	C_2H_5	CH_3	2-Cl	4-Cl
2	CH_3	CH_3	C_2H_5	2-Cl	4-Cl
3	CH_3	CH_3	CH_3	–	4-Cl
4	CH_3	CH_3	CH_3	2-Cl	–
5	CH_3	CH_3	CH_3	–	4-F

Figure 2.5 Chemical structure of organophosphorus compounds [68].

phase from hexane-2-propanol (90:10, v/v) to hexane-ethanol (90:10, v/v). In the case of propiconazole, only three enantiomers could be separated on Chiralcel OD column using hexane-2-propanol (90:10, v/v) with the flow rate of 0.6 ml/min at 15 °C. A satisfactory resolution of difenoconazole was achieved on Chiralcel OJ column using hexane-ethanol (90:10, v/v) as the mobile phase. They had also studied the effects of the temperature with linear Van't Hoff plots at 10–35 °C, and concluded that the enantiomers of these triazole fungicides, with the exceptions of diniconazole and triadimefon, could be separated by altering the temperature [71]. Zhang *et al.* [72] resolved the enantiomers of metalaxyl and metalaxyl acid on Chiralcel OJ-H [cellulose-tris(4-methylbenzoate)] column with *n*-hexane-2-propanol-acetic acid (95:5:0.1, v/v/v) at the 0.5 ml/min flow rate. Zhang *et al.* [73] carried out the chiral separation of three nematicide fosthiazate insecticides with three different polysaccharide chiral stationary phases (Chiralcel OD-H, Chiralpak AD-H, and Chiralpak IB) by HPLC and SFC. In this study, these researchers also observed the effects of the temperature and the organic modifiers on the resolution of analytes and concluded that the best separation of all three analytes was achieved using Chiralcel OD-H column with different ratios of hexane–ethanol as the eluting solvents at the 1.0 ml/min flow rate as shown in Table 2.2. In the case of Chiralcel AD-H and Chiralpak IB columns, all the analytes did not resolve under good conditions with the exception of two compounds that were resolved on both chiral stationary phases. Emerick *et al.* [74] performed the enantioseparation of organophosphorus compound (methamidophos) on four different polysaccharide CSPs – [amylose tris(*S*)-1-phenylethyl carbamate)] as (CSP-1), amylose tris(3,5-dimethylphenylcarbamate) as (CSP-2), cellulose tris(3,5-dimethylphenylcarbamate) as (CSP-3), and the Chiralcel OD [cellulose tris(3,5-dimethylphenylcarbamate)] as (CSP-4) with different compositions of hexane-2-propanol as the mobile phase. A mixture of *n*-hexane-2-propanol (80:20, v/v) was used initially and the chromatographic parameters k, α, and R_s obtained for CSP-1 and CSP-3 indicated poor resolution

with R_s less than 1.0. Furthermore, different ratios of 2-propanol (30, 10, 8, and 5%) were also evaluated with CSP-1, CSP-3, and CSP-4, respectively. Under the chromatographic conditions studied, the highest resolutions R_s were 1.51 and 1.56 when *n*-hexane-2-propanol (95: 5, v/v) and (98: 2, v/v) was used with CSP-3 and CSP-4, respectively. The replacement of 2-propranol by ethanol was also studied and it was concluded that the use of ethanol at 10–20% reduces the retention factor with the poor enantiomeric separation of methamidophos. Similarly, when of *n*-heptane was used instead of *n*-hexane with 1% acid additives, no improvements in the chromatographic parameters for the enantioseparation of methamidophos was observed. Sun *et al.* [75] studied the chiral resolution of uniconalzole and their enantioselective effects on the growth of rice seedlings and cyanobacteria. These authors performed the chiral separation on Chiralpak AD column using *n*-hexane-isopropanol (85:15, v/v) as the mobile phase at the 1.0 ml/min flow rate and the peaks were confirmed with CD and OR detectors at 250 nm as shown in Figure 2.6. Recently, Lao and Gan [76] studied the enantioselective degradation of warfarin in soil samples and resolved the compound with triproline chiral stationary phase and a fluorescence detector. The eluting solvent was *n*-hexane(0.1% TFA)-2-propanol with a ratio of (92:8, v/v) and (96:4, v/v). The baseline separation was achieved using the latter mobile phase.

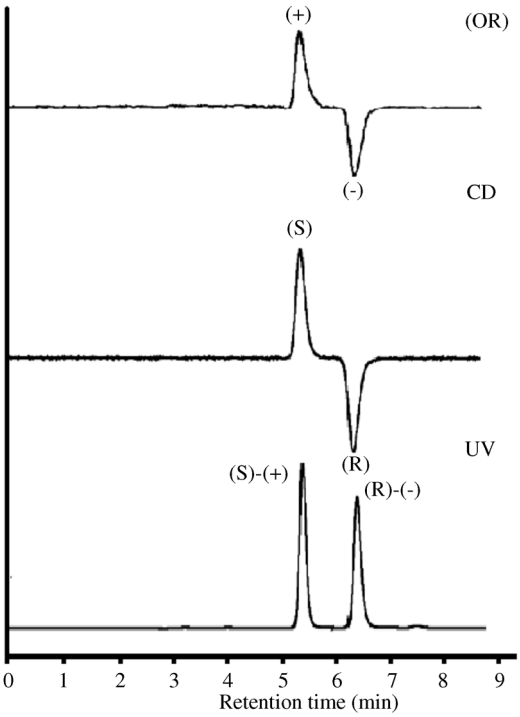


Figure 2.6 Chiral separation of uniconazole on Chiralpak AD column with CD and OR detectors [74].

Wang *et al.* [77] synthesized the chiral selector (amylose tris-(*S*)-1-phenylethyl carbamate) for the enantiomeric resolution of 32 pesticides, in which 10 pesticides showed an interaction with the CSP used, and resolved these pesticides using different compositions of hexane-isopropanol as mobile phases. They also studied the effects of temperature on the resolution and concluded that as the temperature increases, the capacity and separation factors decrease as shown in Table 2.4. Similarly, Tan *et al.* [78] synthesized a new chiral stationary phase

Table 2.4 Effect of temperature on capacity, separation, and resolution factors of pesticides [76].

Pesticides	Temperature °C	Capacity factor		Separation factor α	Resolution (R_s)
		k_1	k_2		
Metalaxyl	0	4.04	6.78	1.68	1.67
	10	3.31	5.42	1.64	2.22
	20	3.01	4.66	1.55	2.51
	30	2.49	3.73	1.50	2.36
	40	2.22	3.19	1.43	2.22
Myclobutanil	0	24.53	31.53	1.29	1.27
	10	20.58	25.91	1.26	1.39
	20	17.49	21.47	1.24	1.49
	30	15.70	19.10	1.22	1.33
	40	14.06	16.85	1.20	1.18
Fenoxaprop-ethyl	0	9.42	12.29	1.30	0.83
	10	6.79	8.92	1.28	0.80
	20	6.07	7.53	1.24	1.02
	30	4.88	5.86	1.20	1.01
Imazalil	40	4.34	5.15	1.19	1.04
	0	9.97	11.25	1.30	0.81
	10	8.61	9.69	1.13	0.89
	20	7.56	8.44	1.12	0.86
	30	6.47	7.16	1.11	0.85
Malathion	40	5.59	6.12	1.09	0.76
	0	6.22	7.10	1.14	0.84
	10	5.05	5.70	1.13	0.80
	20	4.53	5.02	1.11	0.76
	30	3.72	4.03	1.08	0.58
Triadimefon	40	3.24	3.41	1.05	0.44
	0	8.29	12.32	1.49	1.63
	10	6.83	9.81	1.44	1.74

	20	5.97	8.47	1.42	1.84
	30	5.20	7.07	1.36	1.50
Ethofumesate	40	4.83	6.40	1.33	1.36
	0	14.01	15.02	1.07	0.73
	10	11.02	11.78	1.07	0.62
	20	9.95	10.58	1.06	0.63
	30	8.21	8.66	1.06	0.58
Fipronil	40	7.21	7.54	1.05	0.46
	0	10.11	13.09	1.29	0.84
	10	8.33	10.68	1.28	0.92
	20	7.72	9.88	1.28	1.35
	30	6.00	7.64	1.27	1.61
Napropamide	40	4.97	6.28	1.26	1.81
	0	11.16	13.38	1.20	0.97
	10	9.22	10.85	1.18	1.11
	20	7.65	8.76	1.14	1.14
	30	6.77	7.62	1.13	1.04
Paclobutrazol	40	5.54	6.18	1.12	1.07
	0	3.98	4.87	1.22	1.14
	10	3.81	4.64	1.22	1.26
	20	3.57	4.29	1.20	1.30
	30	3.29	3.88	1.18	1.34
trans-permethrin	40	3.06	3.54	1.16	1.31
	15	14.16	15.67	1.11	1.18
	22	12.01	14.60	1.21	1.26
	23	11.01	12.46	1.13	1.34
	25	9.67	11.19	1.16	1.32
	30	7.64	8.88	1.16	1.12
	35	6.64	7.64	1.15	1.02
	38	4.36	4.99	1.14	0.93
cis-permethrin	43	3.27	3.74	1.14	0.78
	15	23.91	26.34	1.10	2.02
	22	23.96	26.47	1.10	2.20
	23	20.57	22.76	1.11	2.21
	25	19.51	21.75	1.11	2.27
	30	17.78	20.27	1.14	2.25
	35	16.83	19.68	1.17	2.31
	38	11.38	14.14	1.24	2.00
	43	7.82	10.26	1.31	1.87

[(*S*)-valine-(*R*)-1-phenyl-2-(4-methylphenyl)ethylamine] for the resolution of pyrethroid insecticides (fenpropathrin, fenvalerate, brofluthrinate, cypermethrin, and cyfluthrin). They also compared it with the resolution on Pirkle-type 1-A chiral stationary phase and achieved a satisfactory baseline separation on the synthesized chiral selector. Oda *et al.* [45] studied the chiral resolution of warfarin on avidin and ovomucoid CSPs using methanol, ethanol, propanol, and acetonitrile as organic modifiers. In general, the chiral recognition behavior of these modifiers on avidin and ovomucoid CSPs had been of the following order: methanol>ethanol>propanol>acetonitrile. Recently, Pan *et al.* [79] compared the enantioresolution of seven triazole fungicides (tebuconazole, hexaconazole, myclobutanil, diniconazole, uniconazole, paclobutrazol, and triadimenol) on the Pirkle-type (*S*,*S*)-Whelk O1 chiral column and four different cellulose derivative columns, namely, cellulose tribenzoate (CTB), cellulose tris-(4-methylbenzoate) (CTMB), cellulose triphenylcarbamate (CTPC), and cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC), in the normal-phase mode with ethanol, *n*-propanol, isopropanol, and *n*-butanol, respectively, as the polar modifier in hexane mobile phase, and concluded that only two triazole fungicides (hexaconazole and triadimenol) had been resolved with different compositions of alcohols in hexane. Among all these cellulose derivative columns, the best separation was achieved on CDMPC and no resolution was achieved on CTB. Some other pesticides were also resolved with different CSPs and mobile phases, and many scholars compared the enantiomeric resolution with various organic mobile phases. The details of the chromatographic parameters are presented in Table 2.2 [77,80,81].

2.4.2
Enantiomeric Separation of Pesticides in a Reversed Phase Mode

The enantiomeric resolution of pesticides using reversed phase chromatography has been carried out using aqueous mobile phases with different nonpolar CSPs. Among all chiral stationary phases, the polysaccharide chiral stationary phases have been widely used as shown in the literature. The majority of the researchers performed the chiral separation of pesticides on polysaccharide CSPs using polar organic modifiers with water or buffers. Ali and Aboul-Enein [60] reported the resolutions of *o*,*p*-DDT and *o*,*p*-DDD enantiomers on Chiralpak AD-RH, Chiralcel OD-RH, and Chiralcel OJ-R chiral stationary phases. The following mobile phases were used: acetonitrile–water (50:50, v/v) and acetonitrile-2-propanol (50:50, v/v) at the 1.0 ml/min flow rate. The detection was carried out at 220 nm for both pesticides. Tian *et al.* [82] developed an HPLC method for the enantiomeric resolution of 20 chiral pesticides on cellulose tris-3,5-dimethyl carbamate (CDMPC) chiral stationary phase under reversed phase mode using different compositions of acetonitrile–water and methanol–water as eluents with the 0.8 ml/min flow rate and the detection at 210 and 230 nm, respectively. The parameters of these findings are presented in Table 2.2. Qiu *et al.* [83] carried out the chiral separation of nine triazole fungicides (hexaconazole, flutriafol,

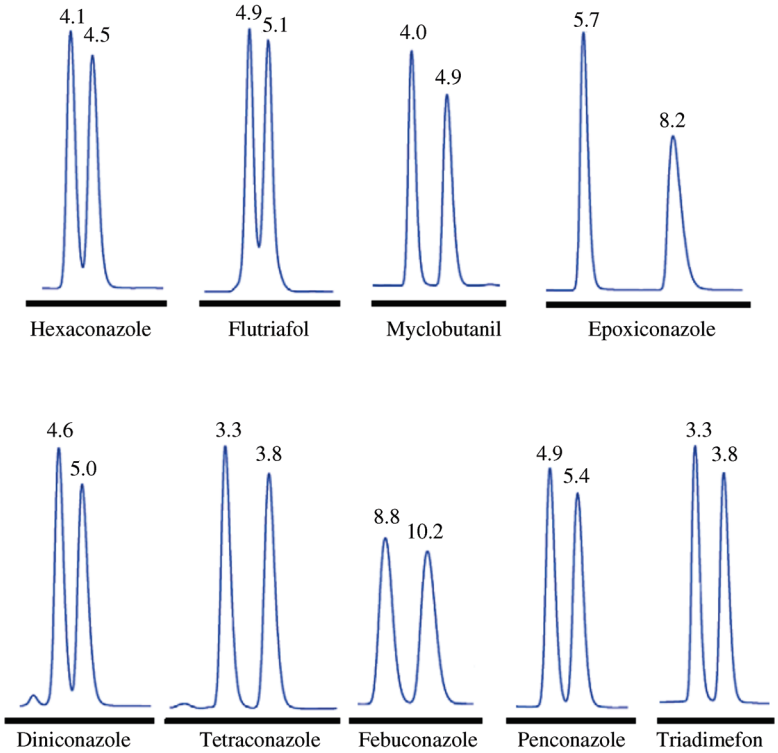


Figure 2.7 HPLC chromatogram of nine triazole fungicides. Mobile phases: methanol/water (75:25) on 3.0 μm column for flutriafol, tetraconazole, penconazole, myclobutanil, fenbuconazole, and triadimefon; acetonirile/water (60:40) on 3.0 μm column for epoxiconazole and acetonitrile/water (60:40) on 5.0 μm column for diniconazole [82].

diniconazole, tetraconazole, epoxiconazole, penconazole, myclobutanil, fenbuconazole, and triadimefon) on the Lux Cellulose-1 columns with different particle sizes (3.0 and 5.0 μm) and with cellulose-tris-(3,5-dimethylphenylcarbamate). In this analysis, the scientists used various ratios of acetonitrile and methanol with water as the organic modifier. Moreover, the effects of temperature on the chiral resolution were also studied. The best separation of all fungicides with different chromatographic conditions is shown in Figure 2.7. Examining this figure makes it clear that the polysaccharide chiral stationary phase is good for the resolution of these fungicides using different ratios of acetonitrile and methanol with water. Li *et al.* [84] also studied the enantiomeric separation of eight triazole fungicides (tetraconazole, fenbuconazole, epoxiconazole, diniconazole, hexaconazole, triadimefon, paclobutrazol, and myclobutanil) in soil samples on polysaccharide chiral stationary phases with the aqueous mobile phase, and a mass spectrometer (MS) detector was used to distinguish the enantiomers. These authors concluded that the best chromatographic separation of all 16 enantiomers of 8 triazole fungicides had been achieved using Chiralcel OD-RH column, with a mixture of

acetonitrile-2 mM ammonium acetate in water (55:45, v/v) as the mobile phase. The baseline separation of all peaks was achieved with more than 1.0 resolution factor R_s. These authors also tested methanol as the organic modifier instead of acetonitrile in reversed phase liquid chromatography. However, unsatisfactory results were obtained. Different concentrations of the ammonium acetate buffer (0.5, 1, 2, 5, and 10 mM) were used to obtain better peaks A good response of the MS/MS detector was observed at the 2 mM buffer concentration. Moreover, other chromatographic conditions were optimized and the best results were obtained at the 0.45 ml/min flow rate at 25 °C. Dong *et al.* [85] performed the enantioselective analysis of myclobutanil (triazole fungicides) in cucumber and soil sample through an LC–MS method. Using this technique, the researchers used the cellulose-based column (Chiralcel OD-RH, 150 mm × 4.6 mm i.d., 5 μm particle size) and the two amylose-based columns (Chiralpak AD-RH and AS-RH, 150 mm × 4.6 mm i.d., 5 μm particle size) with a variety of reversed mobile-phase combinations, and the best chromatographic separation of myclobutanil enantiomers was achieved on Chiralcel OD-RH column with acetonitrile–water (70:30, v/v) as the mobile phase and the 0.5 ml/min flow rate at 40 °C.

Vetter *et al.* [86,87] described the separation of enantiomers of toxaphene on the *tert*-butyl-dimethylsilylated-β-CD-based CSPs using HPLC. Blessington and Crabb [88] performed the chiral separation of aryloxypropionate herbicides on the Chiral AGP column. In this study, the phosphate buffer (10 mM, pH 6)-2-propanol (94:4, v/v) was used as the mobile phase with the detection at 240 nm. Recently, Shishovska and Trajkovska [89] carried out the chiral separation of permethrin enantiomers using the β-cyclodextrin-based chiral stationary phase. The separation of all enantiomers is shown in Figure 2.8. The retention times for the *trans*-enantiomers were 19.8 and 22.7 min, and for the *cis*-enantiomers 38.2 and 42.3 min. They also studied the effects of temperature on the chiral resolution and observed that at high temperatures, the values of chromatographic parameters k, α, and R_s decrease with an increase of temperature as shown in Table 2.4. Schneiderheinze, Armstrong, and Berthod [90] studied the chiral resolution of phenoxyalkanoic acid herbicides in plant and soil samples on Chirobiotic T CSPs with methanol-1% triethylammonium acetate as the mobile

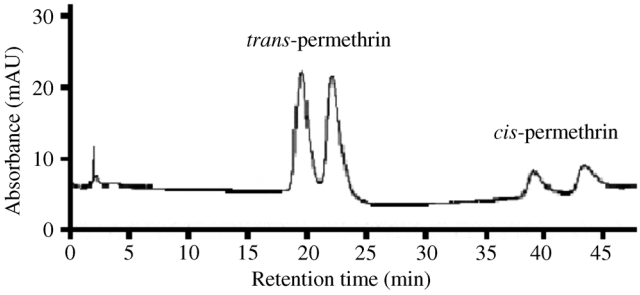


Figure 2.8 Chiral separation of permethrin enantiomers on β-cyclodextrin-based chiral stationary phase [88].

phase at a pH of 4.1 (60:40, v/v), and the detection was carried out by the chiroptical detector. Möller *et al.* [91] resolved the enantiomers of α-HCH in brains of seals on the Chiraldex column using methanol–water (75:25, v/v) as the eluting solvent. Dondi *et al.* [92] used terguride-based CSP for the enantiomeric resolution of chrysanthemic acid [2,2-dimethyl-3-(2-methylpropenyl)-cyclopropanecarboxylic acid] and its halogen-substituted analogs. In this analysis, UV diode array and chiroptical detectors were used for the identification of enantiomers, and it was observed that isomers with (1R) configuration always elute before those with (1 S) configuration. The elution sequence of *cis*- and *trans*-isomers has been strongly affected by the mobile-phase pH, whereas the enantioselectivity has remained the same.

Ludwig, Gunkel, and Hühnerfuss [93] carried out the enantiomeric analysis of 2-(2,4-dichlorophenoxy) propionic acid (dichlorprop) and its degradation products in a marine microbial community on Chiral AGP column with water–2-propanol–phosphate buffer (10 mM, pH 4.85) (94:4: 2, v/v/v) as mobile phase at 230 nm. Armstrong *et al.* [94] developed a method for the enantioseparation of warfarin, coumachlor, coumafuryl, bulan, crufomate, fonofos, anacymidol, napropamide, and 2-(3-chlorophenoxy)-propionamide pollutants on Chiral AGP, and other cyclodextrin-based CSPs. Chu and Wainer [95] separated the enantiomers of warfarin in serum samples using chiral HPLC. Weber, Kreuzig, and Bahadir [96] used the permethylated β-CD CSP for the chiral separation of phenoxypropionates. Recently, Malakova *et al.* [97] studied the chiral resolution of two enantiomers, (*R*)-(+)-warfarin and (*S*)-(−)-warfarin, and their determination in hepatoma HepG2 cell line using a glycopeptide-based chiral stationary phase with the acetonitrile–methanol–ammonium acetate buffer (10.0 mM, pH 4.1) (31:5:64, v/v) mobile phase at 1.2 ml/min flow rate, and the LOD was 0.121 μmol/l for (*S*)-warfarin and 0.109 μmol/l for (R)-warfarin. Guillén-Casla and Pérez-arribas [98] analyzed the enantiomeric separation of mixture of aryloxyphenoxypropionic herbicides (diclofop acid and diclofop-methyl) with α_1-acid glycoprotein CSP using one- and two-dimensional liquid chromatographic methods. In these methods, the achiral separation of samples containing diclofop acid and diclofop-methyl racemate mixtures was carried out by injecting 20.0 μl of sample on the C_{18}-LUNA column. The isocratic mobile phase containing methanol–phosphate buffer (30 mM, pH 7) (73:23, v/v) at the 1.0 ml/min flow rate was used. The chiral resolution of diclofop acid enantiomers and the diclofop-methyl enantiomers was performed with the phosphate buffer (70 mM, pH 7.0)-2-propanol (99.5:0.5, v/v) and the phosphate buffer (30 mM, pH 7.0)-2-propanol (91:9, v/v), respectively, at the 0.8 ml/min flow rate. Recently, the same group of researchers carried out the chiral separation of aryloxyphenoxypropionic acid herbicides (fluazifop-butyl, quizalofop-ethyl, and mefenpyr-diethyl) on the α_1-acid glycoprotein chiral stationary phase. In this study, the optimization of the chromatographic conditions was performed through the factorial experimental design with the phosphate buffer (pH 6.5–7.0) and propanol (5–10%) at the 15–25 °C column temperature. Mathematical deconvolution was also studied to determine peak areas and to calculate R_s, the enantiomeric ratio

(ER), and the enantiomeric fraction (EF), and it was concluded that peak deconvolution provided a simple, effective, and reproducible method for the herbicide determination in soil matrices at low levels of micrograms per gram [99].

Mano *et al.* [100] used the flavoprotein conjugated silica CSP for the chiral resolution of warfarin. They also studied the effects of pH on chiral resolution and observed that the best results were obtained at pH of 4.0–4.8. The effects of different compositions of the mobile phases and column particle size on capacity, separation, and resolution factors under the reversed phase mode using different chiral stationary phases is also presented in Table 2.2 [83].

2.5 Conclusions

The introduction of racemic pesticides into our environment is a serious issue as achiral analyses do not determine the exact dose and toxicity of pesticides. Moreover, different toxicities of the two enantiomers confuse farmers when they measure the dose lethal for pests. Sometimes, the pest control remains ineffective as a result of the illusive dose of racemic pesticides used. Furthermore, it is not possible to determine the impact of the chiral pesticide degradation product without considering the chiral aspect of racemic pesticides. Chiral HPLC methods have proved their effectiveness in the analysis of racemic pesticides and resolution of the corresponding enantiomers. Accordingly, HPLC should be used to study the implications of chiral pesticides before they are introduced into the environment. The enhanced knowledge of chiral pesticides may help determine the required dose and may also save the environment from an unnecessary load of pesticides. Agricultural scientists should think in terms of chiral pesticides and ought to provide information to farmers.

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