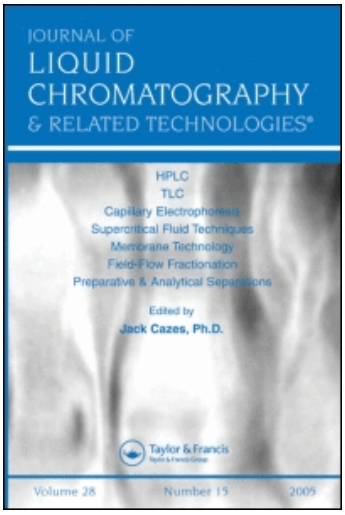

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Practical Guide to HPLC Enantioseparations for Pharmaceutical Compounds

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To cite this Article Thompson, Richard(2005) 'A Practical Guide to HPLC Enantioseparations for Pharmaceutical Compounds', Journal of Liquid Chromatography & Related Technologies, 28: 7, 1215 — 1231
To link to this Article: DOI: 10.1081/JLC-200053033
URL: http://dx.doi.org/10.1081/JLC-200053033

Journal of Liquid Chromatography & Related Technologies®, 28: 1215–1231, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200053033

A Practical Guide to HPLC Enantioseparations for Pharmaceutical Compounds

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Abstract: It is recognized within the pharmaceutical industry that the chiral nature of biosystems results in enantiospecific drug interactions. As a consequence, analytical methods are required to evaluate the enantiomeric purity of active pharmaceutical ingredients. To meet this demand a plethora of chiral selectors have been made commercially available. The options available for a chiral separation are numerous and can be somewhat overwhelming. This review examines some of the more popular chiral stationary phases available for liquid chromatographic separations, provides some insight into their mechanism for enantiorecognition, and provides examples of pharmaceutical ingredients separations.

Keywords: Chiral stationary phase, Pharmaceuticals

INTRODUCTION

The separation of enantiomers is a subject of considerable interest in the pharmaceutical industry as a consequence of the recognition of the stereospecificity of drug-receptor binding. Biosystems tend to be highly stereospecific environments consisting of many chiral components such as proteins, nucleic acids, and polysaccharides. Individual enantiomers of drugs can exhibit different pharmacological, toxicological, metabolic, and pharmacokinetic properties within the chiral environment of these biological systems.[1–10] Guidances by the FDA infer that, whenever practical and economically

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feasible, chiral drugs should be developed as a single enantiomer.[8] If a drug candidate is to be developed as a racemate, it is the responsibility of the drug sponsor to determine the pharmacodynamics, pharmacokinetics, and potentially the toxicology and metabolism of both individual enantiomers along with the racemate. Such requirements in many cases render the development of a racemate economically unfavorable. More than one half of the top 500 drugs are marketed as single enantiomers.[11] The FDA suggests that for drugs developed as a single enantiomer, the stereoisomeric composition should be evaluated in terms of identity and purity. The undesired enantiomer should be treated as a structurally related impurity and its level be assessed by an enantioselective means.

There are a number of techniques available for the evaluation of enantiomeric drug purity. Techniques not requiring separation include polarimetry,[12] nuclear magnetic resonance using shift reagents,[13–15] enzyme reactions,[16] circular dichroism,[17] and enantioselective sensors.[18] Separation techniques include chromatography and electrophoresis and can be performed directly or through a precursory derivatization with a chiral agent.[19] Direct separations require the formation of energetically distinct transient diastereomers with a chiral agent attached to a stationary phase or contained within the mobile phase. This review will focus on direct liquid chromatographic separations where the chiral agent is attached to the stationary phase. There are more than 100 commercially available chiral stationary phases (CSPs). With the tremendous amount of publications relating to chiral separations, a comprehensive review is not feasible. Rather, this review will focus on the more popular chiral stationary phases.

The model for interaction of an enantiomeric drug with a CSP to form a transient diastereomeric complex invokes the "three point interaction" rule.[20] This rule stipulates that at least one of the enantiomers must be capable of undergoing a minimum of three simultaneous interactions with the CSP and that the overall interactions of the two enantiomers with the CSP must be energetically distinct. The types of interactions that can occur include hydrogen bonding, charge transfer, dipole interactions, ionic interactions, and steric interactions. These interactions can be attractive or repulsive. The predominant types of interactions that occur are dependent upon the functional groups present on the solute, the mobile phase, and the chiral stationary phase.

CSPs range from small bonded synthetic selectors to large biopolymers. The small bonded synthetic selectors are characterized by well defined molecular structures and are designed for specific types of interactions. In many cases the binding energy of these types of phases to a particular analyte can be predicted through molecular modeling.[21] Thus the elution order for an enantiomeric pair can also be predicted. At the other end of the spectrum are the biopolymers which are constructed from chiral sub-units and are thus intrinsically chiral. Elucidation of the interactions of a solute with these types of phases are more complex due to the multitude of chiral

sites and the role of the tertiary structure of the biopolymer in chiral recognition.[22,23] This review will focus on the major classes of CSPs that are most commonly utilized in the pharmaceutical industry. They are Pirkle type, ion pair, cyclodextrin, crown ether, protein, polysaccharide, and antibiotic phases.

PIRKLE TYPE PHASES

The Pirkle type phases are generated through the binding of a low molecular weight selector to a silica or zirconia substrate. They were introduced by William Pirkle who has been a significant innovator and whose work has provided tremendous insight into the mechanisms of chiral recognition. The individual chiral selector molecules are evenly distributed onto the silica substrate and most often act independently in their interactions with the solute. These phases show good mass transfer, chemical and thermal inertness, and are compatible with most mobile phases. In many cases it is possible to predict the elution order of enantiomers using these types of phases as a consequence of the relatively small size of the selector and selectand.

The Pirkle type phases are amino acid derivatives possessing an aromatic entity which can undergo $\pi-\pi$ interactions with the solute. The aromatic entity can either be a π donor or π acceptor. The CSP and the solute form a π donor/acceptor pair. This complex is then stabilized by additional interactions such as hydrogen bonding, dipole interactions, or steric repulsion. Pirkle initially utilized a number of N-(3,5-dinitrobenzoyl) amino acids as CSPs.[24,25] Stationary phases with phenylglycine and leucine as the amino acid moieties are commercially available. These π-acceptor phases were then followed by π-donor phases utilizing a naphthylamnio acid functionality.[26,27] Alanine and valine based phases are commercially available. The commercially available Whelk-O1 columns possess both dinitrobenzoyl and naphthyl moieties.[28] Elution order is controlled by choice of the stereochemical configuration of the CSP as both enantiomers of the selector are marketed.

The Pirkle type phases have been used to separate a large number of pharmaceutical compounds. The phases are most commonly used in normal phase mode in order to enhance the $\pi-\pi$ and hydrogen bond interactions.[29–32] Hexane with an alcoholic modifier, such as isopropanol, is the mobile phase of choice. Separations of non-steroidal anti-inflammatory drugs,[28] antiepileptics,[25] and benzodiazepinones[33] have been performed under normal phase conditions.[28] These phases have also been utilized in the reversed phase mode[34] with poorer enantioselectivity and in some cases different elution order indicating a change in the chiral recognition mechanism. These phases can also be utilized in super/sub- critical conditions.[35–40] Antimalarials,[35] β-agonists,[40] and β-blockers[36,40] have been separated in this mode.

ION PAIR PHASES

Chincona alkaloids such as quinine and quinidine are effective counter-ions for carboxylic and sulfonic acids. These alkaloids have been previously used as mobile phase additives and as CSPs under normal phase mode for enantiomeric separations.[41–45] Chincona alkaloid derived carbamates immobilized to silica, marketed as ProntoSIL Chiral AX, demonstrate good enantioselectivity to chiral acids.[46,47] Both quinine and quinidine are semi-rigid structures possessing 5 stereogenic centers. Despite being diastereomers and not enantiomers, quinine and quinidine derived CSPs frequently produce opposing elution orders of enantiomers.[46] This fact indicates that the stereogenic centers where quinine and quinidine differ may be the major sites for chiral recognition. Potential interaction sites include the protonated basic aliphatic nitrogen of the quinuclidine ring through ion pairing, the carbamate moiety for hydrogen bonding, the π-basic quinoline ring for $\pi-\pi$ interactions, and both ring structures for steric interaction.[46]

While these phases show good enantioselectivity in the normal phase mode there are more commonly utilized in the reversed phase mode to take advantage of ion-pair interactions. These phases are consequently classified as weak chiral anion exchangers.

Like other weak anion exchangers, retention is maximized where the product of ionized selector and selectand concentration is greatest. Consequently the working pH range tends to be between 4 and 7 for the separation of most carboxylic acids. Ibuprofen and naproxen were resolved on these phases in reversed phase mode.[46] The hydrolysis product and synthetic precursor of the calcium antagonists, clevidipine and felodipine, exhibited good enantioseparation under polar organic phase mode using a mobile phase of 0.125% acetic acid in acetonitrile.[48] These phases have also separated neutral racemic imidazo-quinazoline-dione derivatives.[49] For these separations and for separations under normal phase conditions there are no ion pair interactions and the interactions are anticipated to be similar to those found with the Pirkle type phases.

CYCLODEXTRIN PHASES

Cyclodextrins are natural macrocyclic polymers of glucose. They have been used extensively for chiral separations. The α, β, and γ- cyclodextrins contain 6, 7, and 8 glucose units respectively. The cyclodextrins are chiral toroidal shaped molecules with an overall appearance of a truncated cone. They possess relatively non-polar cavities which allow for inclusion of non-polar moieties of guest molecules through hydrophobic interactions. Additional interactions can also occur through hydrogen bonding between the guest molecule and the hydroxyl groups at the lip of the cyclodextrin or

through steric hindrance. The hydroxyl groups of the cyclodextrins can be modified by derivatization to form alkyl, hydroxyalkyl, acetyl, aryl carbamate, or other functionalities. Cyclodextrins or derivatized cyclodextrins are extensively utilized in capillary electrophoresis and as mobile phase additives in liquid chromatography for chiral separations. In addition, columns where cyclodextrins have been covalently bonded to a silica substrate are commercially available (CycloBond). These columns can be used in normal phase, reversed phase, polar organic phase, and sub/supercritical phase mode.

An inclusion mechanism occurs in the reversed phase mode and is strongest with increasing water content of the mobile phase. The selectivity of the inclusion complexation is dependent upon the hydrophobicity and steric compatibility of the enantiomers. Separation is favored for molecules possessing aromatic and hydrogen bonding moieties close to the stereogenic center and where the stereogenic center is in a rigid environment such as a ring.[50,51] In the reversed phase mode it is advantageous to change pH or the ionic strength of the mobile phase to enhance the inclusion mechanism. Changing the pH may reduce the net charge of the solute and increase it hydrophobicity while increasing the ionic strength will enhance hydrophobic interactions. Common buffers include triethylamine phosphate, triethylamine acetate, and ammonium acetate. Methanol or acetonitrile are the preferred organic modifiers. A number of pharmaceutical compounds have been resolved using β-cyclodextrin columns in reversed phase mode including methadone, anticonvulsants, calcium channel blockers, β-adrenergic blockers, antiinflammatories, β-2 receptor agonists, anti-cancer agents, dihydrofurocoumarins, and antidepressants.[52–62]

In other separation modes, the solute has to compete with the non-polar components of the mobile phase for the cavity of the cyclodextrin and the recognition mechanism changes. In the polar organic mode the dominant interactions are hydrogen bonding with the hydroxyl groups on the exterior of the cyclodextrin. Separation is favored for solutes with multiple hydrogen bonding moieties and bulky groups near the stereogenic center. The mobile phase in the polar organic mode is commonly acetonitrile with small amounts of methanol to control retention. In some cases the use of triethylamine acetate may be required to enhance resolution. Enantiomeric separations of β-blockers, anticholinergics, non-steroidal anti-inflammatories, diuretics, anxiolytic agents, and proglumide have been demonstrated in the polar organic mode.[62–65]

The most useful cyclodextrins used in normal phase and sub/supercritical modes are derivatives of naphthylethylcarbamate or dimethylphenylcarbamate allowing for $\pi-\pi$ interactions along with hydrogen bonding.[66] Hexane modified with an alcohol such as isopropanol in the normal phase mode, and carbon dioxide modified with alcohol in the sub/supercritical mode are commonly utilized. A diverse group of drugs including

ancymidol, bendroflumethiazide, benzoin, cromakalin, ibuprofen, mephenytoin, piperoxan, tropicamide, verapamil, proglumide, and suprofen have been enantiomerically resolved in the super/sub critical mode.[64,67]

CROWN ETHER PHASES

Crown ethers are heteroatomic macrocycles possessing a hydrophobic exterior and a hydrophilic cavity. The cavity of crown ethers has a strong affinity for cations predominantly through electrostatic interactions. The 18-crown-6 ethers can complex not only inorganic cations but also protonated primary amines. This inclusion interaction occurs primarily through the formation of a triple hydrogen bond between the hydrogen atoms of the ammonium group and the oxygen atoms of the crown ether. The same complex is formed whether the guest approaches from the top or the bottom of the crown ether. The introduction of bulky groups onto the exterior of the crown ethers provide steric barriers and induce enantioselective interactions with the guest molecule. If the guest molecule is capable of forming reasonably stable complexes with the crown ether, then repulsive/attractive interactions with the barriers lessens the stability of the complex of one enantiomer relative to the other resulting in chiral discrimination.

Crown ethers dynamically coated onto an octadecylsilyl substrate was found to exhibit good chiral recognition for amino acids.[69,70] This crown ether, with phenylnaphthyl barriers, was subsequently marketed commercially by Daicel under the name of CrownPak CR. Both enantiomers of the crown ether are available allowing control of the elution order of the enantiomers. Because the crown ether is dynamically coated the column is susceptible to extensive bleeding when organic eluents are utilized. The manufacturer recommends that no greater than 10% methanol be used with these columns. Consequently these columns can only be used for relatively hydrophilic solutes. Eluents of 0.1% acid are a good start point for development. At this pH the amine should be protonated. Retention is affected by the choice of acid. The counter anion of the more chaotropic acids such as perchloric acid increases retention relative to that of the less chaotropic phosphoric acid.[70] However, there is usually negligible effect on enantioselectivity as a function of the acid used.[70] Retention can also be controlled as a function of the counter ion concentration. Increased concentrations of the counterion in the mobile phase result in increased retention, but again with negligible changes in enantioselectivity.[70] At high pH values, as the silica substrate becomes deprotonated, additional electrostatic interactions between the protonated solute and the deprotonated silanol sites can lead to increased retention and peak broadening. Recently, successful enantioseparations of racemic amines, amino alcohols, and α-aminocarbonyl compounds were performed on a CSP of phenylnaphthyl crown ether covalently bonded to silica.[71]

A second commercially available crown ether column, RCA/SCA marketed by Regis, is made by covalently binding 18-crown-6-tetracarboxylic acid to a silica substrate. The presence of carboxylic acid groups provides another parameter other than steric hindrance to influence enantioselectivity. Above pH 2, these carboxylic acid groups can deprotonate leading to electrostatic interactions between the crown ether host and the amine guest. Strong acids will suppress the ionization of the carboxylate groups on the crown ether reducing the electrostatic interactions with the amine solute and consequently its retention. Silanophilic interactions will be strong above pH 3 leading to increased retention. Since the crown ether is covalently bonded there is greater flexibility for using organic solvents in the mobile phase. An increase in organic modifier concentration may lead to increased retention[72] due to increased electrostatic and hydrogen bonding interactions as the mobile phase becomes less polar. Both enantiomers of the CSP are available allowing for control of elution order of the enantiomers. These CSPs were found to also separate the enantiomers of secondary amines through a combination of hydrogen bonding and electrostatic interactions.[73]

ANTIBIOTIC PHASES

The antibiotic glycopeptides, vancomycin, teicoplanin, and ristocetin A, have been extensively utilized as chiral selectors.[74–76] These macrocyclic antibiotics possess several characteristics that enable them to stereoselectively interact with solutes. They contain an aglycon bucket consisting of 3 or 4 macrocyclic rings. They also possess multiple stereogenic centers and a number of functional groups including sugars, aromatic rings, phenol groups, amide linkages, amine and acid/esters moieties. As a consequence, they can interact with a solute through hydrogen bonding, dipole interactions, $\pi-\pi$ interactions, hydrophobic interactions, electrostatic interactions, and steric hindrance.[77–80] Antibiotics were introduced as chiral selectors by Armstrong et al.[74] and have been subsequently utilized as chiral selectors in capillary electrophoresis and liquid chromatography. The three antibiotics are commercially available as Chirobiotic CSPs. There are two types of teicoplanin stationary phases, one with the sugar moieties removed, and they exhibit different enantioselectivity. The columns can be used in normal phase, reversed phase, polar organic and sub/supercritical modes. These columns show very good selectivity to amino acids and other carboxylic acids but also resolve many neutral and basic solutes.

In the reverse phase mode, the optimum operating pH range for these columns is between 4 and 7. Triethylammonium and ammonium acetate buffer are common aqueous components of the mobile phase with acetonitrile, methanol, or THF as the organic component. Electrostatic interactions along with hydrophobic interactions are anticipated to be the dominant interactions.

Proglumide, profens, temazepam, verapamil,[74] calcium channel modulators,[81] barbiturates, mephenytoin, thalidomide[82] have been enantiomerically resolved in reversed phase mode with the antibiotic columns.

In the polar organic mode, methanol or acetonitrile are commonly utilized as the eluent. The predominant interaction is also believed to be electrostatic interactions. However hydrophobic interactions are weaker and hydrogen bonding and dipole interactions are enhanced relative to the reversed phase mode. Triethylammonium acetate is often used as an additive to ionize the stationary phase and the solute. These phases have been used to enantioseparate antidepressants,[83] albuterol,[84] potential local anesthetics,[85] and vesamicol.[86] in the polar organic mode.

In the normal phase mode, a hexane/alcohol mobile phase is often utilized. Trifluoroacetic acid and triethylamine are generally utilized as additives to ionize the stationary phase and the solute. These additives can also be utilized to reduce secondary non-specific interactions. The antibiotic phases have been used to enantioseparate barbitals, propranolol,[74] calcium channel modulators,[81] and barbiturates[82] in the normal phase mode.

In the sub/super critical mode a carbon dioxide/alcohol mobile phase is commonly utilized. Trifluoroacetic acid and triethylamine are generally utilized as additives. The interactions that occur under these conditions are similar to those in the normal phase mode. However, the sub/super critical phase mode generally results in greater peak efficiency due to the higher diffusivity and lower viscosity associated with the eluent. Benzodiazipines, β-blockers, β-agonists, barbiturates, and non-steroidal anti-inflammatories[87–90] have been resolved with these CSPs in this mode. It should be noted that the ristocetin CSP was unable to resolve the enantiomers of the tested β-blockers. This lack of chiral recognition may be attributed to the lack of a carboxylic moiety on this CSP that can undergo electrostatic interactions with the β-blockers.

PROTEIN PHASES

Proteins are polypeptides with typically 100–1000 chiral amino acid residues. There are 20 different amino acids from which proteins are made. These polypeptide chains are not bunched up in random masses. Rather, they assume recognizable patterns including helices, reverse turns, and pleated sheets. The combined patterns dictate the tertiary structure of the protein. In a polar environment the protein assumes a configuration such that its surface is polar and non-polar residues are buried in the interior of the structure. The complex structure of the protein bio-polymers renders elucidation of mechanisms for chiral recognition to be a much more difficult task than for the low molecular weight chiral selectors. However, it is believed that chiral recognition occurs predominantly though hydrophobic interactions in an apolar

calyx that is buried in the interior of the structure.[91–93] In the calyx, additional interactions such as electrostatic interactions, hydrogen bonding, dipole interactions, and steric hindrance occur.

A number of proteins are commercially available as CSPs including α-acid glycoproteins (AGP, the major plasma binding protein for basic drugs), human serum albumin (HSA, the major plasma binding protein for weakly acidic drugs), bovine serum albumin (BSA), ovomucoid (OVM), and cellobiohydrolase (CBH). The proteins are bonded to silica and utilized in reversed phase mode with an aqueous buffer/organic modifier eluent. Mobile phase optimization is performed through variation of the pH, ionic strength, temperature, and organic modifier. Using BSA or AGP phases, positively charged solutes such as amines tend to be more strongly retained at higher pHs and negatively charged solutes tend to be more strongly retained at lower pH.[94,95] This effect reflects the increased hydrophobicity of the solute allowing for its partitioning into the apolar calyx. Enantioselectivity can also change as a function of pH. A reversal of elution order was observed as a function of pH.[96,97] Increased ionic strength of the buffer also enhances the hydrophobic interaction and thus increases retention.[94,95] Increased amount of organic modifier reduces retention.[94,95] The nature of the organic modifier also influences enantioselectivity. Reversal of elution order has been observed depending upon the organic modifier used.[96,98–100] This effect most likely reflects a change in the tertiary structure of the protein. Temperature also affects enantioselectivity. Generally enantioselectivity increases with decreasing temperature. Reversal of elution order has also been observed as a function of temperature.[100,101] The protein CSPs are very broad based in the types of drugs that they can enantioseparate relative to the smaller molecular weight selectands. They are numerous separations cited for the separation of a diverse group of basic, acidic, and neutral drugs.[99,103–133]

POLYSACCHARIDE PHASES

The polysaccharides are naturally occurring condensation polymers of sugar. Amylose and cellulose are two of the most common polysaccharides occurring in nature. They differ in the nature of their linkage. At the supramolecular level, the polysaccharides contain ribbon like chains that are held together by intra- and inter- molecular hydrogen bonding that result in the formation of sheets. Successive sheets are held together by van der Waal interactions. Each sugar unit possesses five chiral centers and the lamellar arrangement of the polymer provide a multitude of chiral cavities which impart chiral character at the supramolecular level. The native polysaccharides are not practically useful for CSPs due to their weak resolving ability and mechanical weakness. However derivitization, through reaction at the active hydroxyl

groups, results in improved chromatographic and enantioselective properties. The first derivatized CSP was triacetylcellulose.[134] Phenylester and phenylcarbamate derivatives of cellulose and amylose are currently the most commonly utilized stationary phases. The difference in linkage of cellulose and amylose results in different spatial arrangements of the chiral cavities. A combination of NMR, x-ray analysis, and molecular modeling indicate that cellulose triphenylcarbamate possesses a left handed three fold helical conformation with the glucose residues regularly arranged along the helical axis. The corresponding amylose derivative possesses a left handed four fold helix.[135–137] A chiral helical groove runs along the main chain. Aromatic groups are located on the surface of the chain and the less polar carbamates are located in the interior.[137] Furthermore, the shape of the chiral cavities varies depending upon the presence of an ester or carbamate group.

Chiral recognition on polysaccharide phases are attributed to shape selective inclusion into the chiral grooves enhanced by additional interactions such as hydrogen bonding, dipole interactions, π interactions, and van der Waal forces depending upon the chromatographic mode. Enantioselectivity can vary as a function of amylose versus cellulose, ester derivative versus carbamate derivative, mobile phase components, temperature, and chromatographic mode.

Several variations of the triphenylesters and triphenylcarbamates of amylose and cellulose are commercially available from Diacel. These phases show the broadest applicability of all of the commercially available CSP and are capable of resolving a large and diverse selection of chiral solutes. The more popular phases are the 3,5, dimethylphenylcarbamates of amylose and cellulose (Chiracel AD and Chiralpak OD respectively). For most of these phases, the polysaccharide is dynamically coated onto a silica substrate. A 3,5- dimethylphenylcarbamate derivative of amylose that is covalently bonded to silica was recently introduced (Chiralpak IA). The polysaccharide phases are very flexible in that they can be used in normal phase, reversed phase, polar organic, and sub/supercritical mode.

In the normal phase mode, chiral recognition occurs through steric fit into the chiral cavities enhanced by hydrogen bonding, π-interactions, and dipole interactions. An eluent of hexane/2-propanol is commonly utilized. Other alcohols can be utilized and the structure of the alcohol influences the enantioselectivity.[138–140] Aprotic solvents such as acetonitrile, methyl tert-butyl ether, methylene chloride, ethyl acetate, or THF can give superior separations where hydrogen bonding is a strong contributor to chiral recognition.[141] Though not recommended by the manufacturers, these mobile phases have been used with a dynamically coated Chiracel OD column with no stability problems.[141] These solvents can also be used with the covalently bonded Chiralpak IA. For the analysis of basic pharmaceuticals, the use of an amine additive such as triethylamine, diethylamine, or diisoprpoylamine is recommended to reduce peak tailing.[142,143] For acidic pharmaceuticals, the use of an acidic additive such

as trifluoroacetic acid can be used to improve peak shape and decrease retention.[143,144] There are numerous citations of chiral separations for pharmaceutical compounds in the normal phase mode.[145–157] In the sub/supercritical mode, the chiral recognition is similar to the normal phase mode with the added advantage of higher diffusivity and lower viscosity of the eluent. A large number of pharmaceutical compounds have been resolved under sub/supercritical conditions.[158–169]

There is a burgeoning usage of polysaccharide phases in the reversed phase mode. The dominant chiral recognition method is believed to be shape specific inclusion into the chiral cavities.[170] Given the polarity and hydrogen bonding capabilities of the conventional eluents, secondary interactions between the solute and the CSP, such as hydrogen bonding, are weakened relative to the normal phase mode. The reversed phase mode is useful for solutes which cannot be analyzed under normal phase conditions due to insolubility or unfavorable retention characteristics. A simple water/alcohol or acetonitrile eluent can be utilized for neutral solutes as these separations appear to be insensitive to electrolytes in the eluent.[171] For acidic compounds optimal retention and selectivity is usually observed at low pH. Unlike the protein columns, the polysaccharide phases do not contain ionizable groups. At low pH, the ionization of acid solutes is suppressed allowing for greater interaction with the CSP. For basic compounds, the presence of a chaotropic counteranion allows for greater retention and selectivity.[171] Counteranions such as perchlorate, hexafluorophosphate, and tetrafluoroborate are recommended. A large number of reversed phase separations of pharmaceutical compounds have been reported.[170–187]

Though not as popular, separations can also be performed in the polar organic mode. Typical eluents are pure acetonitrile, methanol, ethanol, acetonitrile, and their mixtures. Selectivity is different from the reversed phase mode. This mode is very popular for preparative separations because of the high solubility of polar solutes in these eluents and the simplicity of removing the eluents by evaporation.[188] A number of polar organic separations have been reported.[189–196]

SUMMARY

A large number of chiral stationary phases are currently available to meets the needs of the pharmaceutical industry for determination of the enantiomeric purity of active pharmaceutical ingredients, raw materials, and metabolites. As a consequence, there are a multitude of options in terms of columns, separation mode, and separation conditions to explore in achieving an enantioseparation. In fact the proliferation of columns and separation options may appear somewhat overwhelming at first glance. Chirbase (http://chirbase.u-3mrs.fr/chirbase), a database specializing in chiral chromatographic

separations, lists over 100,000 separations. This database indicates that polysaccharide based stationary phases are the most frequently utilized phases accounting for ~ 40% of the separations.[197] This data base can be searched for separations of structurally related compounds to provide some guidance to the analytical chemist. Strategies have also been developed using a knowledge based system for method development in normal and reversed phase mode starting with three of the polysaccharide phases.[198] Hopefully this review has also offered some insight into the available options for development of chiral methods.

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Received October 10, 2004
Accepted December 14, 2004
Manuscript 6549F