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Review

Enantiomeric Resolution of Drug Compounds by Liquid Chromatography

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Novel techniques have recently emerged to separate chiral drug compounds into pure enantiomers. The mechanism, experimental difficulties, and applicability of these methods can vary greatly, and the choices involved are not straightforward. The most significant new advances in the field of chiral separations have come from work done with liquid chromatographic systems and chiral stationary-phase columns. This review describes several commonly used approaches to chiral separation, diastereomeric derivatization, chiral mobile-phase additives, and three major types of chiral stationary phases. Although no single method can be judged superior for every drug application, it appears that chiral stationary phases have received the most attention recently and they are emphasized here.

KEY WORDS: chiral separations; separation of optical isomers; high-performance liquid chromatography (HPLC); chiral stationary phases; diastereomeric derivatization.

INTRODUCTION

The resolution of enantiomers (nonsuperimposable mirror-image isomers) from racemic mixtures of pharmaceutical compounds has become one of the most extensively studied disciplines within pharmaceutical analysis. Most biological substances exist in only one enantiomeric form, whereas chiral drug compounds are usually formulated and administered as the racemic mixture. Further, racemic drug mixtures may display different pharmacological effects for the individual enantiomers. Frequently, one of the enantiomers is less active than the other, and undesired toxic effects may be caused by one optical isomer and not the other. For example, *d*-thalidomide provides the desired hypnotic effect, whereas *l*-thalidomide is responsible for the well-known teratogenic effect (1).

The ability to separate enantiomers of drugs is important in both industrial pharmaceutical analysis and biopharmaceutical analysis. The synthesis of optically pure drugs is difficult, and traditional methods of resolving racemic mixtures are inefficient. The pharmaceutical industry has therefore become interested in the chromatographic resolution of enantiomers on a preparative scale as a means of producing optically pure dosage forms. In cases where it is less difficult to synthesize individual enantiomers, analytical-scale separations are needed to establish the optical purity of reaction products. Biopharmaceutical separations of enantiomers (separations of enantiomers isolated from biologic fluids) are necessary for the analysis of differences in physiologic disposition which may occur between enantiomeric pairs. More specifically, differences in metabolism between enantiomeric pairs can be evaluated by analysis of the enantiomers of drug metabolites.

High-performance liquid chromatography (HPLC) has become the method of choice for the separation of enantiomers. This is because of the wide applicability of the method and the speed and efficiency at which these separations can be carried out (2). Gas chromatography has also been used for this purpose, with the major limitation of being restricted to volatile drugs or drug derivatives (3). Also, the absorptive differences between enantiomeric pairs become quite small at high temperatures, which generally result in less efficient separation. High temperatures can also cause racemization of chiral stationary phases and possibly the analyte.

There are many different mechanisms by which enantiomers can be separated by HPLC. Chiral recognition can occur through precolumn derivatization with chiral reagents, the addition of chiral reagents to the HPLC mobile phase, and selective interactions with chiral stationary phases. Each of these general methods has a unique set of advantages and limitations. These are discussed along with specific mechanisms involved. Application of these methods to drug production and analysis is also presented.

DERIVATIZATION USING CHIRAL DERIVATIZING REAGENTS

A carbon or other atom to which four different groups are attached represents a chiral center. A molecule is designated as an R or S isomer depending on whether substituent groups are sequenced clockwise (R) or counterclockwise (S) with regard to decreasing molecular weight. Enantiomeric compounds have one chiral center and exist as mirror

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images of the alternate enantiomer. Diastereomers are compounds having more than one chiral center and are not mirror images. Diastereomers have similar chemical properties but different physical properties. Enantiomers have identical chemical and physical properties except for the direction of rotation of plane polarized light.

Resolution of enantiomers by chemical conversion to diastereomers followed by chromatographic separation was first developed using gas–liquid chromatography (GLC) (4). Although many successful GLC methods have been developed for the resolution of drugs, this method suffers from the limitations already discussed. Most new methods developed for resolution of optical isomers therefore use liquid chromatography as the mode of separation. Initial work on the mechanisms responsible for these separations was done by Krager and Pirkle (5,6). This method of resolution of enantiomers has been well summarized by several authors (7–10).

Separation Mechanism

The resolution of enantiomeric mixtures by this method is based on the difference in physical properties of diastereomers. When a racemic mixture is reacted with a single, pure enantiomer of the chiral derivatizing reagent (Fig. 1), diastereomers are formed. The reaction involves either covalent or ionic bond formation. Chiral derivatizing reagents forming covalent bonds are far more frequently used than chiral ion-pairing agents. These diastereomers, once formed, can be separated by conventional liquid chromatographic techniques, e.g., normal or reversed-phase chromatography. Since the enantiomers are converted to diastereomers, then analyzed, this method is referred to as the indirect method. In addition to the need for enantiomeric purity, chiral derivatizing reagents should be capable of reacting with the target functional group readily and selectively.

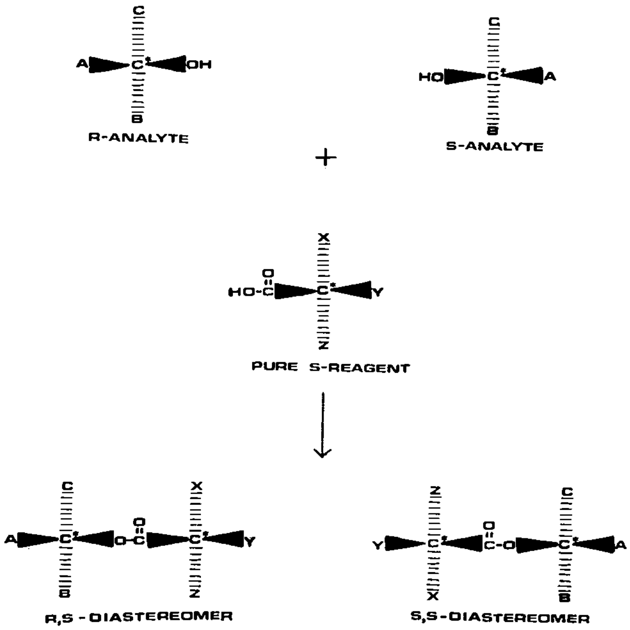

Fig. 1. Configural representation of the reaction between a racemic mixture and a pure chiral derivatizing reagent.

Chromatographic Conditions

The diastereomers formed may be separated by reversed or normal-phase chromatography. This requires no special considerations other than those necessary for classical separations based on differences in partition or adsorption.

Pharmaceutical Applications

There are many asymmetric drugs containing either an amino, a hydroxyl, or a carboxylic acid group, which can be easily attached to chiral reagents. A number of acids or derivatives thereof (chlorides, anhydrides, imidazolidides) have been used to prepare diastereomeric amides with racemic mixtures of amines. Two commercially available isocyanates have been extensively used for resolution of β-amino alcohols from the β-adrenergic antagonists class of drugs (11–13). An isothiocyanate derivative has been used to resolve racemic mixtures of propranolol and several of its analogues (14). The acid chloride of *l*-methoxyacetic acid has been used in the resolution of various enantiomeric drugs (15,16). Nonsteroidal antiinflammatory drugs of the 2-aryl propionic acid type can be esterified with optically active alcohol, and a number of alcohols including (*d*)-2-octanol (17) and *l*-2-butanol (18) have been used for derivatization.

Advantages

There is a wide variety of potential derivatization reagents available, and this method has a broad scope of application. A review by Gal cites 36 chiral derivatizing agents that have been used (9). Since diastereomers are separated by conventional chromatographic conditions, the method does not require special equipment. Finally, in weakly detectable compounds the sensitivity may be enhanced by choosing a reagent that is a strong chromophore or fluorophore.

Limitations

Derivatization is a precolumn synthetic process thus inhibiting the use of automated procedures. Enantiomeric purity of the derivatizing reagent is absolutely essential, and the chiral reagent is required to be stereochemically stable under storage conditions. The reaction between the two enantiomers and the chiral derivatizing reagent may not proceed at the same rate. This may generate different proportions of the two diastereomeric products (19). The chiral derivatizing reagents should react under sufficiently mild conditions to avoid significant degradation of the reactants. The chiral derivatizing reagents are not always easy to produce and may be expensive. It is also not possible to recycle chiral derivatizing reagents.

CHIRAL MOBILE-PHASE ADDITIVES

Diastereomer complexes can be formed by adding a chiral agent to the mobile phase which interacts with the analyte. Racemic mixtures are resolved because of differences in stabilities of the diastereomeric complexes, their solubility in the mobile phase, or the degree of binding of the complexes to an achiral stationary phase. Lindner and Petterson have recently reviewed this method (9).

Various mobile-phase additives have been used to form diastereomeric complexes. Transition metal complexes (chiral ligand-exchange chromatography) were first used by Karger and others (20,21). Chiral ion-pair chromatography has been reviewed by Petterson and Schill (22). Cyclodextrins have also been used as mobile-phase additives which achieve chiral separation through inclusion complexation.

Separation Mechanism

Diastereomeric tertiary complexes are formed between a transition metal, a single enantiomer of a chiral molecule, and a racemic mixture of the analyte. The various transition metal ions show the following order of decreasing efficiency: Cu(II), Ni(II), Zn(II), and Cd(II) (23). The selector ligand is usually an amino acid, e.g., *l*-proline (24), *l*-aspartylcyclohexamide (25), *l*-arginine, *l*-histidine (26), or *l*-2-isopropyl-4-octyl-diethylenetriamine (23). In an aqueous mobile phase containing the *l*-amino acid (*l*-Pro) and Cu(II) at a 2:1 ratio the following equilibrium can exist with the enantiomeric mixture of the analyte (R-A/S-A).

$$[l\text{-Pro–Cu–}l\text{-Pro}]^- + \text{R-A}^- \rightarrow [l\text{-Pro–Cu–R-A}]^- + l\text{-Pro}^-$$
$$[l\text{-Pro–Cu–}l\text{-Pro}]^- + \text{S-A}^- \rightarrow [l\text{-Pro–Cu–S-A}]^- + l\text{-Pro}^-$$

Resolution of R and S isomers would be achieved when one of the ligands forms a stronger mixed complex. Stereoselective association between ions has been used in chiral ion-pair chromatography to resolve enantiomeric compounds. Diastereomeric ion pairs are formed between a charged solute and a chiral counterion of opposite charge. These ion pairs have structural differences such that they show different chromatographic properties (22). The various counterions that have been employed are *d*-10-camphorsulfonic acid (22), quinine, quinidine, cinchonidine, *d*-di-*n*-butyl tartarate, albumin (27), pectic (polygalacturonic) acid (28), DEAE-Sephadex, and QAE-Sephadex (29). When albumin is used, stereoselective retention seems to be possible only if the racemic compound has one or two negatively charged groups, an aromatic ring system, and a polar function in the vincinity of the chiral center (27).

Resolution of enantiomers based on the formation of inclusion complexes can be due to two processes (30). The racemic mixture may be resolved due to a difference in the absorption of the diastereomeric complexes on the achiral stationary phases or the resolution may be based on the relative stabilities of the diastereomeric complexes.

Chromatographic Conditions

Aqueous mobile phases are used with the transition metal complex method. The separation of isomers is optimal in the proline system when the concentration of acetonitrile is 20% (26). A marked increase in retention with increasing pH is observed (23), corresponding to an increase in selectivity.

Separation with mobile-phase additives is generally based on the properties of ion pairs, so systems favoring a high degree of ion-pair formation have been used, i.e., mobile phases of low polarity such as methylene chloride. The retention of the solute can be decreased by increasing the concentration of the counterion or by the addition of a polar modifier (*l*-pentanol or isopropanol) to the mobile phase. Water, even at very low concentrations, appears to have an adverse influence on separation (22). Aqueous mobile phases containing phosphate buffer can be used when employing albumin as the counterion. Both aqueous and nonaqueous mobile phases can be used with *d*-di-*n*-butyl tartarate. Cyclodextrins can be used with aqueous buffers and retention can be reduced by adding alcoholic modifiers to the mobile phase (31).

Pharmaceutical Application

Transition metal complexes have been used primarily to separate racemic mixtures of amino acids (20,21). There has been some success with other classes of compounds including carboxylic acids, amino alcohols (as Schiff bases), barbiturates, hydantoins, and succinimides. Alprenolol, oxprenolol, metaprolol, propranolol, and hyoscyamine have been successfully resolved using *d*-10-camphorsulfonate (22). Enantiomers of carboxylic acids have been separated with albumin, and amines would also be suitable analytes. Mephenytoin, methyl phenobarbital, and hexobarbital, in addition to mandelic acid and some of its derivatives, have been resolved using β-cyclodextrins (31).

Advantages

Chiral ligand exchange is an excellent method for resolution of amino acids and amino acid-like compounds. No precolumn derivatization is required. Aqueous mobile phases can be used, and automated column switching techniques can be applied easily. Preparative-scale resolution of racemic mixtures is possible with β-cyclodextrins.

Limitations

Resolution of enantiomers is limited to α-amino or α-hydroxy carboxylic acids by using transition metal complex. However, primary alcohols have been derivatized to the salicylaldehyde Schiff base and resolved with chiral *l*-proline Cu(II) complex. Additives are costly or must be synthesized. The chiral ion pairs sometimes may not be stable and water is usually detrimental to the system. Changes in pH, ionic strength, and temperature can affect the chromatography, making this approach difficult. Sensitivity of the method is generally reduced due to interference by counterion absorbance in the UV region. Application of cyclodextrins as mobile-phase additives is limited, since appropriate spatial configuration is important for inclusion-complex formation.

CHIRAL STATIONARY PHASES

Pirkle Type

Pirkle-type stationary phases are based on selective interactions (π–π bonding, dipole, hydrogen bonding, and van derWaals interactions) between a chiral stationary phase and individual enantiomers (33,34). These stationary phases are the benefit of work done by William H. Pirkle and co-workers and have resulted in the introduction of the first commercially available HPLC column containing a chiral stationary phase (35). Since the initial work of Pirkle, there have been a number of different stationary-phase designs which are based on Pirkle's original principle (36,37), and a number of different classes of compounds have been separated (38–41).

Mechanism. Chiral recognition is achieved through the formation of transient diastereomer-like complexes between the analyte and the stationary bonded phase. Three points of interaction are believed to be required for selectivity between pairs of enantiomers. This three-point recognition concept is shown in Fig. 2, where a pure enantiomeric bonded-phase molecule (R) differentially interacts with the (R) and (S) forms of the analyte molecule. It can be seen from Fig. 2 that the (R) form of the analyte provides three points of interaction (hydrogen bonding between carbonyl oxygen on the stationary phase and the hydroxyl group on the analyte, van derWaals interaction between alkyl groups, and π-bond donor/acceptor interaction between the aromatic group on the analyte and the electron withdrawing group on the chiral stationary phase), whereas the S enantiomer is capable of only two (the interaction between alkyl groups is prevented due to the spatial orientation of the groups). The R-form interaction is therefore stronger and would result in a more stable complex. This enhanced stability produces larger retention volumes, which result in the desired separation.

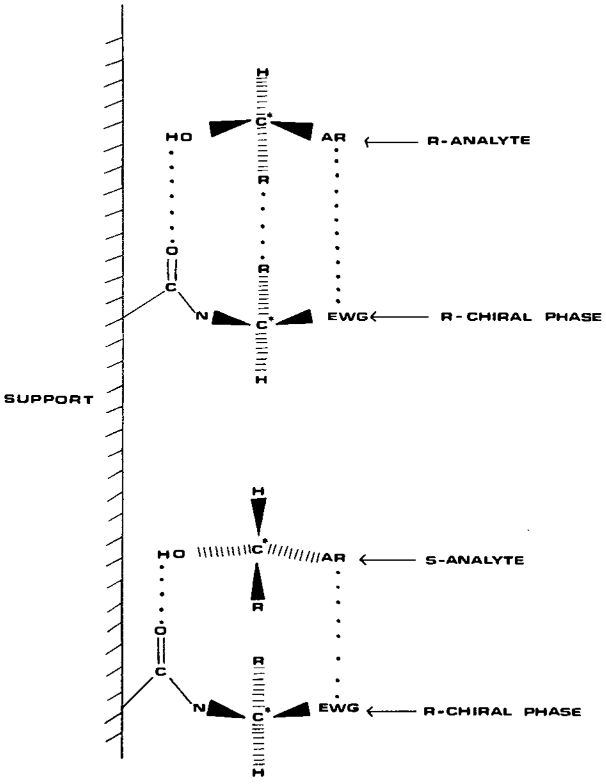

Fig. 2. Configural representation of the three-point interaction model for chiral recognition. AR, aromatic group; R, alkyl group; EWG, π-electron withdrawing group (usually dinitrobenzene analogues).

Chromatographic Conditions. Pirkle-type chiral columns are generally used in the normal phase mode. Mixtures of hexane modified with isopropanol to adjust retention are the most commonly used mobile phases and the covalently bonded-type column can be used with small amounts of very polar solvents such as water. The selectivity and stability of these columns appear to be much better with hydrophobic mobile phases, however, and these are recommended (42). Enantiomers of organic acids and bases are poorly resolved by Pirkle-type columns and are usually derivatized to form corresponding esters (40) and amides (41).

Pharmaceutical Applications. There are many different varieties of Pirkle-type stationary phases to achieve the specific interactions mentioned above. Both π-electron acceptor and π-electron donor groups are used to interact selectively. Examples of some types of compounds separated by π-electron acceptor stationary phases include benzyl alcohols, propranolol analogues, aryl substituted lactams, substituted hydantoins, benzodiazepinone, gluthethimide, amphetamine derivatives, and α-aryl acetamides (43–47). The π-electron donor type has been used to separate the enantiomers of amines, amino acids, alcohols, and thiols, although an achiral derivatization step is required.

Advantages. A primary advantage of the Pirkle-type column is its broad applicability. These columns are also relatively efficient, with efficiencies of about 60,000 theoretical plates/m routinely obtainable with covalently bonded-phase Pirkle columns (48). Enantiomeric selectivities (α values) of greater than 100 have been observed (49), and preparative-scale separations have been reported (50). Pirkle-type columns have been used for the enantiomeric analysis of propranolol and gluthethimide in biologic fluids (44,45). A wide variety of Pirkle-type columns are commercially available. There has been a larger variety of compounds separated by this mode than any other chiral stationary phase (3). The elution order of enantiomers is also more predictable by this method.

Limitations. Traditional Pirkle-type stationary phases are not compatible with aqueous mobile phases. This limits utility with regard to biologic fluid analysis and coupled-column techniques. Covalently bonded columns can be used with polar mobile phases, although selectivity and column stability are sacrificed (3). Precolumn derivatization is often required for acids and bases. These derivatization agents are achiral, however, and the need for pure enantiomeric reagents is obviated.

Inclusion Complexation

Inclusion complexes are entities comprising two or more molecules, in which one of the molecules, the "host," includes, by physical forces, a "guest" molecule. Cyclodextrins (CyD) are typical host molecules. The first effective use of CyD in chromatography was as mobile-phase modifiers in thin-layer chromatography (2). Prior to this, polymerized CyD gels were used as stationary phases in column chromatography with varying degrees of success (51,52).

CyDs are prepared by enzymatic degradation of prehydrolyzed starch. They are cyclic oligosaccharides linked by α-glycosidic bonds (2,51–53). There are three forms of CyDs, α, β, and γ, which contain six, seven, and eight glycopyranose units respectively. The structure of β-Cyd is shown in Fig. 3. The present commercially available CyD stationary phases have a seven- to nine-atom spacer between the support and the CyD molecule (53). The physical shape of the molecule is that of a truncated cone, with an internal cavity whose dimensions are determined by the number of glucose units (54). The internal diameter and other dimensions are shown in Fig. 4.

Separation Mechanisms. Due to the uniform stereochemistry of the glucopyranose units, all secondary hydroxyl groups are situated on one of the two edges of the CyD ring, whereas all the primary hydroxyl groups are on the other edge. The inner cavity is lined by the glycosidic oxygen bridges and is hydrophobic. The orientation of hydroxyl groups is such that the C2 hydroxyls are located in a clockwise direction, and the C3 hydroxyls in a counterclockwise direction. Thus the hydroxyl groups of adjacent glucopyranose units form hydrogen bonds which stabilize the shape of the molecule (55).

The β-CyDs have been more widely applied because of their optimum size for inclusion-complex formulation. Each glucose unit contributes five chiral centers, thus the β-CyD molecule contains 35 chiral centers. Guest solutes can interact through van derWaals forces if the guest is of suitable size to fit tightly within the β-CyD cavity.

If the enantiomer has the suitable polar substituent(s), one or more favorable hydrogen bonds can form (56). The attainment of enantioselectivity appears to require three points of interaction between the enantiomer and the β-CyD (56,57). At least one substituent of the guest must be tightly interacting with the CyD cavity. In addition, the arrangement of appropriate substitutents bonded to an asymmetric atom must be in close proximity to the CyD rim hydroxyl groups so that two other points of interaction can occur (55). These findings are consistent with the three-point attachment concept introduced by Dalgliesh (58) and has been well studied by Armstrong *et al.*, who used computer-generated projections of the lowest free energy inclusion complexes of R- and S-propranolol (59). Inclusion-complex formation requires that a compound have at least one aromatic ring. In addition, close proximity of the ring moieties to the chiral center appeared to improve chiral resolution.

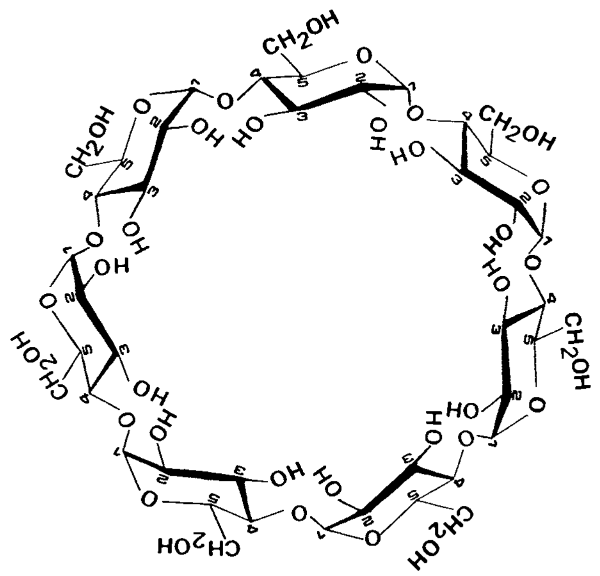

Fig. 3. Chemical structure of the β-cyclodextrin molecule.

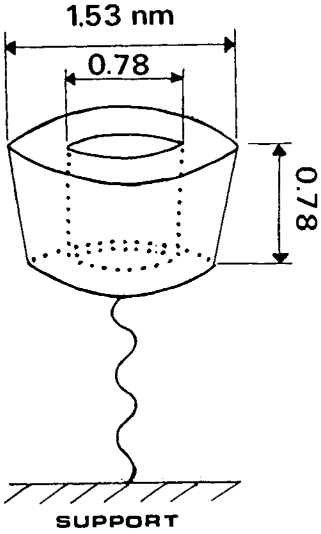

Fig. 4. Representation of the size and shape of the β-cyclodextrin molecule as it exists attached to a solid support.

Chromatographic Conditions. An important distinction of CyD columns is that separations are most often accomplished in the reversed-phase mode (water/alcohol mobile phases). The organic phase modifier tends to compete with all solutes for the preferred location in the hydrophobic cavity. Consequently, increasing the concentration of organic modifier will decrease retention. Similarly, a solvent modifier that forms a stronger complex will also reduce retention at a lower relative concentration, e.g., ethanol binds more strongly to CyD than methanol (60,61). Generally, the binding strength of ionic species to the CyD is less than for the corresponding neutral species. Accordingly, control of pH (within the allowable range of 4.0 to 7.5) can be used to affect the course of a separation (60). Band broadening and long retention can be reduced by increasing the column temperature (62). At temperatures above 50°C, however, the stationary phase is susceptible to decomposition.

Pharmaceutical Applications. Satisfactory resolution has been obtained for the enantiomers of methadone, verapamil, metoprolol, aminogluthethamide, and nisolidipene using CyD-bonded phases (59). The separations were reproducible during the 4 months that columns were used, and preparative-scale separations were achieved (59). Diastereomeric pairs of cardioactive and antimalarial cinchona alkaloids can be easily separated, and separation of the geometric (*cis* and *trans*) isomers of the antiestrogens has also been accomplished (63).

The absence of differential interaction at the mouth of the CyD cavity may preclude chiral recognition in some cases. It may be possible to circumvent this problem by forming derivatives of the C2 or C3 hydroxyl groups which are located at the tip of the cone. For example, stereoisomers of *d*-norgestrol could be separated with an acetylated β-CyD column but not with an underivatized one (60).

Previous work by Hayashi *et al.* showed that inclusion complexes are formed with E-, A-, and B-type prostaglandins (55). The number of theoretical plates for the separation was approximately 30,000/m. The authors found that γ-CyD provided lower retention than the β-CyD column, confirming that the physical size of the hydrophobic cavity is important in the separation of isomers. (64).

Advantages. Aqueous mobile phases can be used without damaging the column and derivatization of the solute is generally not required. The technique is well suited for automated column-switching techniques and suitable for the preparative resolution of racemic mixtures. Another benefit of CyD columns is that they can be used for achiral separations as well, which may preclude the need for column-switching techniques (3).

Limitations. The major drawback with this method is the strict structural requirement for chiral recognition. Since the molar volume of the β-CyD cavity is only approximately 346 Å^3 (54), only those guest molecules of appropriate size and shape can form strong inclusion complexes. This can be overcome to a certain extent by using γ-CyD bonded-phase columns. The formation of strong inclusion complexes results in somewhat broad band widths, although the small loss of efficiency is more than offset by the gain in selectivity.

Protein Type

The interaction between acidic and basic drug compounds and proteins is well known. The proteins involved are albumin, which is the primary binding protein for acidic drugs, and α-1-acid glycoprotein (AGP), which binds primarily basic drugs. These proteins are polymers composed of naturally occurring chiral amino acid subgroups and the binding that occurs can often be stereospecific. Allenmark *et al.* (65) and Hermansson (66) have developed bonded-phase columns that take advantage of the albumin and AGP interactions, respectively. Both types of columns are now commercially available and a number of drug enantiomers have been separated by this technique.

Mechanism. The differential retention of enantiomers by protein columns is not clearly understood. The separation appears to take place through differential interaction of enantiomers by a combination of hydrogen bonding and van derWaals mechanisms (48). It has also been proposed that ion pairing (67) and charge transfer (68) interactions may be contributory. The fact that protein binding sites are selective with regard to enantiomers is not surprising, however, since receptors (69) and enzymes (70) have been shown to handle individual enantiomers quite differently.

Chromatographic Conditions. Separation is achieved with protein columns through careful adjustment of the pH, ionic strength, and organic modifier concentration of the mobile phase (71). The columns are used in the traditional reversed-phase mode with aqueous buffered mobile phases containing various amounts of 2-propanol, which is the usual organic modifier. The pH range over which these columns are stable is 3.0–7.5, and ion supression effects which would be expected for reversed-phase chromatography are observed (71). Separations on AGP columns are sensitive to both temperature and flow rate, with results typical of reversed-phase HPLC. Retention has been shown to increase faster than resolution for disopyramide enantiomers when the temperature is decreased (1). This may indicate that temperature control is not a very effective way of controlling separation. Increasing the flow rate produces a large decrease in resolution and flow rate should not exceed 0.5 ml/min for column stability purposes. Adjusting flow, therefore, is also not an effective way of achieving optimum separation. The effects of increasing the organic modifier concentration and increasing the ionic strength are to decrease and increase the retention volumes, respectively. The effect of the organic modifier concentration is much more pronounced (1).

Pharmaceutical Applications. The primary use of protein columns has been in the stereospecific separation of drug compounds. This is exemplified in a review by Wainer *et al.* (71), where AGP columns were shown to resolve successfully the enantiomers of 50 commonly used drugs. Although racemic mixtures of drugs can often be resolved directly, it has been shown that derivatization increases selectivity in some cases (72). Enantiomeric separations with protein columns is not limited to acids and bases and studies by Schill and co-workers have suggested that close proximity of large structures to the chiral binding site may be important in achieving stereospecific separations (71).

Advantages. The primary advantage of protein columns is their wide applicability to enantiomeric separations of drug compounds. The analysis of drugs from biologic fluids is made more simple by the fact that aqueous mobile phases are used. The approach is flexible in terms of separation variables so that parameters may be adjusted to achieve the desired separation. Obviation of derivatization reagents is another potential advantage of protein columns.

Limitations. Because of the limitations put on flow, protein columns generally require more time than other types of chiral phase separations. This results in broader peaks and poor sensitivity, which may limit use for low-dose drugs in biologic fluids. Another major limitation of protein columns is their low capacity. This suggests that enantiomeric separations on a preparative scale may not be practical by this approach.

CONCLUSIONS

The separation of enantiomers is one of the most challenging tasks in separation science. Although chiral separations are subject to the same general restrictions as conventional HPLC, the very specific nature of the interactions involved requires very careful optimization of parameters to achieve enantiomeric selectivity.

The need for preparative-scale separations within the pharmaceutical industry is evident because synthesis of pure enantiomers is often not economically feasible. Preparative-scale separations generally require greater selectivity than is currently achieved by the various enantiomeric separation approaches. The problem is, therefore, not one simply of scale but of increasing enantiomeric selectivity. This may be accomplished through new and innovative approaches to perform enantiomeric separations or by combining existing approaches. It is clear that this technology is not far from being widely integrated into drug development and will undoubtedly be increasingly more integrated into regulatory aspects of drug production and marketing. Leaders in this technology will most likely be leaders in new product development of chiral drug compounds as well.

The stereospecific analysis of drugs and their metabolites in biologic fluids will undoubtedly yield results that demonstrate the need for pure enantiomeric dosage forms. These types of analyses not only will require attention to enantiomeric selectivity but also will require robust methods that are sensitive and selective with regard to biological contaminants. Achiral separation ability such as that demonstrated by cyclodextrin columns becomes important with regard to selectivity in the biological matrix. Compatibility with automated column-switching technology may also be important in this regard. Sensitivity may be either enhanced or lessened by derivatization and must therefore be considered for biologic fluid analysis. To avoid band broadening with an accompanying loss of sensitivity, columns must be efficient or concentration of the eluted peaks must be considered.

None of the methods available for enantiomeric separations can currently be regarded as the method of choice for all types of drug separations. Diastereomer derivative formation and the addition of chiral reagents to mobile phases will probably becomes less popular because of the need for optically pure reagents. Chiral stationary phases will be chosen, with attention to the fact that different compounds may be more appropriately separated on different stationary phases and with different goals in mind. The technology of each method is progressing such that it is unlikely that one method would emerge as generally superior to the rest for drug enantiomer separations. Until that time, the choice is primarily empirical, and it is clear that this will remain an area of stimulating research in the near-future.

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