



CHIRAL DRUG ANALYSIS AND THEIR APPLICATION

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Accepted on: 06-12-2010; Finalized on: 10-02-2011.

ABSTRACT

The biological activity of chiral substances often depends upon their stereochemistry, since the living body is a highly chiral environment. A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show significant enantioselective differences in their pharmacokinetics and pharmacodynamics. Two main approaches to chiral drug analysis have been taken. In the so-called indirect approach, the drug enantiomers are derivatized with an optically pure chiral reagent to form a pair of diastereomers, which may then have sufficiently different physicochemical properties for separation to occur on conventional chromatographic columns. In the direct approach, transient rather than covalent diastereomeric complexes are formed between the drug enantiomers and a chiral selector present either in the mobile or the stationary chromatographic phase. In the current context there is looking for advances stationary phases like Whelk-O[®] 1, Leucine, 3-Gem 1, Pirkle 1-J, DACH-DNB, ULMO, Chiral AGP, Chiral CBH, Chiral HAS, CHIRALPAK IA, NUCLEOCEL DELTA, CHIROBIOTIC, macrocyclic glycopeptides phases etc. Chiral analysis of biomolecules like amino acids, sugars, proteins, lipid and nucleic acids are interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. Along with this other important application of chiral drugs analysis are therapeutic drug monitoring, in clinical and forensic toxicology and chiral specific applications of essential oils, flavors, and pharmaceutical drugs.

Keywords: Chiral drugs analysis; chiral stationary phase; enantiomers.

INTRODUCTION

Chiral compound

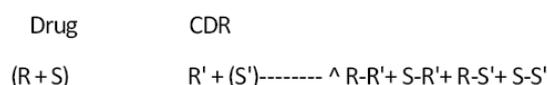
Any carbon atom that is bonded to four different functional groups is termed a chiral or an asymmetric carbon. Molecules containing one or more of these carbon centers are considered chiral molecules. Chiral centers can exist in two forms called enantiomers. These two forms are non-superimposable mirror images of each other, but both have similar properties. For example, both enantiomers will have the same boiling point, densities, and reaction rates as a chiral molecule. They do, however, generally possess different aroma and flavor characteristics; more importantly, they possess differences in toxicity and biological activity. Two main approaches to chiral drug analysis have been taken. In the so-called indirect approach, the drug enantiomers are derivatized with an optically pure chiral reagent to form a pair of diastereomers, which may then have sufficiently different physicochemical properties for separation to occur on conventional chromatographic columns. In the direct approach, transient rather than covalent diastereomeric complexes are formed between the drug enantiomers and a chiral selector present either in the mobile or the stationary chromatographic phase. Alternately, the chiral selector may be provided by the spatial configuration of L-amino acids in an antibody binding site, thus forming the basis for competitive binding enantioselective immunoassays. Each of these analytical approaches has advantages and disadvantages^{1,2}.

Indirect chiral drug analysis

The success of this approach depends on the availability of stable, optically pure chiral derivatizing reagents (CDR) and of course on the presence of suitable functional groups in the chiral drug molecule for covalent formation of diastereomeric derivatives. The reaction scheme may be illustrated as follows:



The necessity for high optical purity and stability of the CDR may be illustrated by a consideration of the reaction products from a racemic drug and the R-enantiomer of a CDR which is contaminated with its S-antipode:



In this case, an additional pair of diastereoisomers is formed (R-S', S-S') each of which is the enantiomer of one of the first pair. Thus, the enantiomers R-R', S-S' and S-R', R-S' would coelute in conventional chromatographic systems. Such contamination (or racemization during the reaction) would lead to analytical error and this would be especially critical when attempting to quantitate small quantities of one enantiomer in the presence of a large excess of its antipode. For most pharmacokinetic studies, optical contamination of the CDR of up to 1% is tolerable. Precautions must also be taken to avoid the possibility of "kinetic resolution" of drug enantiomers resulting from their differential reaction rates with the CDR.



The main advantages of this approach are the following.

- A large variety of reagents and reactions are available permitting diastereomeric derivatives with good separation and detection possibilities to be formed.
- The proper selection of the homochiral derivatisation agent makes it possible to select the advantageous elution order of the peaks: in possession of both the (*R*) and (*S*) forms of the derivatisation agent it is achievable that the minor peak of the enantiomeric impurity elutes before the main peak thus improving its quantitation.
- It is possible to use inexpensive a chiral stationary phases.
- The major advantage of the indirect technique is that conventional chromatographic columns (GC; normal and reversed-phase HPLC) may be utilized for the separation of the diastereomers.

Thus, considerable flexibility in chromatographic conditions is available to achieve the desired resolution and to eliminate interferences from metabolites and endogenous substances. Moreover, a reasonably good selection of chemically and optically pure CDRs is available for derivatizing various functional groups.

Selected applications of the indirect technique for chiral drug analysis in biological fluids include the gas chromatographic resolution of methamphetamine stereoisomers in urine after derivatization with *N*-trifluoroacetyl-L-prolyl chloride, and the use of (*S*)-*cr*-methoxy-*a*-trifluoromethylphenylacetyl chloride for the GC chiral analysis of tocainide enantiomers in plasma. Applications employing a chiral HPLC columns include the analysis of propranolol and 4-hydroxypropranol enantiomers in serum after derivatization with (+)-(*R*)-phenylethylisocyanate, and the use of (-)-camphanoyl chloride as a CDR for the chiral analysis of racemic proxyphylline in plasma.

Several reviews are available in the literature summarising the main features of the very high number of (usually commercially available) chiral derivatising agents and reactions³⁻¹⁴. The reagents include acyl chlorides and anhydrides (e.g. (*S*)-(+)-naproxen chloride), chloroformates (e.g. (—)-menthyl chloroformate and (+)-1-(9-fluorenyl)ethyl chloroformate), isocyanates (e.g. (/?)-*a*-methylbenzyl isocyanate), isothiocyanates (e.g. 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate) and others (e.g. 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide, *o*-phthalaldehyde + *N*-acetyl-L-cysteine) for the derivatisation of *amines* and *amino acids*, acyl chlorides, anhydrides and cyanides (e.g. (ft)-(+)-*a*-methoxy-*a*-trifluoromethyl-phenylacetyl chloride, (/?)/(?)*O*,*O*-diacetyltartaric anhydride and (—)-2-methoxy-1,1'-binaphtha-lene-2-carbonyl cyanide) for *alcohols* and *phenols* and amines after the activation of the COOH group (e.g. 1,1-carbonyldiimidazol + (*S*)-(—)-*α*-methylbenzylamine) for carboxylic acids.

In the overwhelming majority of cases the diastereomers are separated by high-performance liquid

chromatography. Especially in the early literature gas chromatographic separations can also be found (e.g. *N*-heptafluorobutyryl-L-prolyl chloride for the separation of the enantiomers of amphetamines¹⁵). HPLC¹⁶ and thin-layer chromatography¹⁷ are equally suitable for the separation of the enantiomers of chiral carboxylic acids after their derivatisation with (1/?2/?)-(4-nitrophenyl)-2-amino-1,3-propanediol or its enantio-mer using dicyclohexylcarbodiimide as the activator of the carboxyl group. Although most of the vast quantity of papers published on this topic deal with the general and theoretical problems of the enantiomeric separation and with the analysis of biological samples, in principle the majority of the described methods could be suitable for the determination of the enantiomeric purity, too. As for the sensitivity attainable the method of Demian and Grisphover¹⁸ for the determination of (7?)*-*3-aminoquinuclidine in the (*S*)-enantiomer and *vice versa* can be regarded to be characteristic; as little as 0.1% enantiomeric impurity could be determined after derivatisation with (i?,i?)*-*0,0-dibenzoyl-tartaric acid anhydride and separation on a C₈ column with as short an analysis time of 3 min.

Although the other possibilities for enantiomeric separations, discussed in the subsequent part of this section seem to be more generally used, new derivatisation reagents are time to time introduced up to the present time. Examples for this are 4-(3-isocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazol and 4-(3-isocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulphonyl)-2,1,3-benz-oxa-diazol¹⁹. These reagents are available both in the *R* and *S* form and excel with their high enantiomeric stability and the mild conditions for the derivatisation reaction with amines to form diastereomeric thiourea derivatives, which can be well separated on C₁₈ columns.

Another derivatisation reaction which merits special mentioning is the enzyme-catalysed glucuronidation of hydroxy compounds. The high enantiomeric purity of the reagent ((+)-uridine 5'-diphosphoglucuronic acid), the high selectivity and very mild conditions of the reaction catalysed by the 5'-diphosphoglucuronyltransferase enzyme and the excellent separation of the diastereomeric derivatives by RP-HPLC make this method eminently suitable for enantiomeric purity check as exemplified with the study of the drug candidate (-)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin (enantiomeric purity 99.89%) and its (+)-enantiomer (99.84%)²⁰. As it has been mentioned an advantage of diastereomeric derivatisation is that it enables nonchiral columns to be used. However, if the separation power of a chiral column is combined with the good separability of the diastereomeric derivatives, very delicate problems can be solved as demonstrated by Matuszewski *et al*²¹. The four stereoisomers of 1[(—)-*SS*-4-ethylamino-5,6-dihydro-6-methyl-7,7-dioxide-4H-thieno(2,3b)thiopyran-2-sulphonamide and its four deethylated stereoisomeric derivatives, among them metabolites, heat and light



degradation products were separated on a Pirkle-type chiral column after the derivatization of the ethylamino or amino groups with (S)-(+)-1-(1-naphthyl)ethyl isocyanate to form the diastereomeric urea derivatives.

Christian Roussel et al. Worked on semi-preparative resolution of the atropisomers by chiral liquid chromatography and determination of the barriers to rotation has allowed an unequivocal identification of the regioisomers produced by the reaction between *N*-(2-methoxyphenyl)-*N'*-(2-methylphenyl)thiourea and α -chloroacetone.

Direct chiral analysis

In the direct approach transient rather than covalent diastereomeric complexation occurs between a chiral selector and the analyte. Discrimination of enantiomers is considered to depend on a three-point interaction between one enantiomer and the chiral selector. At least one of these interactions must be stereochemically dependent such that the other enantiomer can only form a less stable two-point complex. The chiral discriminator may be present in the mobile phase for use with conventional HPLC columns or it may be incorporated into the stationary phase to provide specialized chiral stationary phases (CSPs). A clear advantage of the direct method is that reaction with a CDR to form diastereomers is not required. Nevertheless, derivatization may still be necessary, but with a non-chiral reagent, in order for appropriate molecular interactions with the chiral discriminator to occur and/or to impart requisite spectral or fluorescent properties to the molecule.

Although much progress has been made in elucidating chiral recognition mechanisms, this knowledge in many cases are insufficient to allow prediction of which chiral selector is best suited to achieve a desired chiral resolution. Thus, some trial and error is to be anticipated. Moreover, mobile phase flexibility is limited for some CSPs. Thus, it may be difficult to resolve drug enantiomers in biological fluids from metabolites or endogenous substances. In some instances, coupling an achiral column in series with a chiral column was required to eliminate such interferences.

MORDEN CHIRAL STATIONARY PHASES (CSPS)

Pirkle Stationary Phases

Whelk-O[®] 1 (Analytical to Preparative Columns) is useful for the separation of underivatized enantiomers in a number of families including amides, epoxides, esters, ureas, carbamates, ethers, aziridines, phosphonates, aldehydes, ketones, carboxylic acids, alcohols and non-steroidal anti-inflammatory drugs (NSAIDs). This π -electron acceptor/ π -electron donor phase exhibits an extraordinary degree of generality. The broad versatility observed on the Whelk-O 1 column compares favorably with polysaccharide-derived chiral stationary phases. In addition, because Whelk-O 1 is covalently bonded to the support, the phase is compatible with all commonly used mobile phases, including aqueous systems — a distinct advantage over polysaccharide-derived chiral stationary phases. Other advantages include column durability, excellent

efficiency, ability to invert elution order and excellent preparative capacity.

Whelk-O[®] 2 (Analytical to Preparative Columns) is the covalent trifunctional version of the Whelk-O 1. The Whelk-O 2 retains the same chiral selector but incorporates a trifunctional linkage to the silica support. Whelk-O 2 was designed to improve the resistance of the stationary phase to hydrolysis while using strong organic modifiers such as trifluoroacetic acid. The Whelk-O 2 is ideal for preparative separations since the material is bonded on 10 μ m, 100Å spherical Kromasil silica. This allows the preparative chromatographer to perform method development on an analytical column and immediately scale up to larger diameter columns.

Leucine (Analytical and Semi-Preparative Columns) The π -acceptor leucine CSP is based on 3,5-dinitrobenzoyl leucine, covalently bonded to 5 μ m aminopropyl silica. Columns derived from either L- or D-leucine are available. This phase demonstrates enhanced enantioselectivities for several classes of compounds, including benzodiazepines.

Phenylglycine (Analytical and Semi-Preparative Columns) Phenylglycine, a π -acceptor chiral phase, is based on 3,5-dinitrobenzoyl phenylglycine, covalently bonded to 5 μ m aminopropyl silica. Phenylglycine columns are available in both L- and D- configurations. This CSP resolves a wide variety of compounds containing β -basic groups, including: aryl-substituted cyclic sulfoxides, bi-P-naphthol and its analogs, α -indanol and α -tetralol analogs, and aryl-substituted hydantoins.

3-Gem 1 (Analytical and Semi-Preparative Columns) is a π -acceptor chiral stationary phase and is prepared by covalently bonding N-3,5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)-propanoate, to 5 μ m silica through an ester linkage. It can separate anilide derivatives of chiral carboxylic acids, including nonsteroidal anti-inflammatory agents.

α -Burke 2 (Analytical and Semi-Preparative Columns) phase is derived from dimethyl N-3,5-dinitro-benzoyl- α -amino-2,2-dimethyl-4-pentenyl phosphonate covalently bound to 5 μ m silica. This π -acceptor chiral stationary phase is particularly valuable in the HPLC separation of (3-blocker enantiomers, an important class of cardiovascular drugs whose enantiomers often exhibit differing pharmacological activities.

Pirkle 1-J (Analytical and Semi-Preparative Columns) is the latest in a series of CSPs from the research laboratories of Professor Pirkle. This new CSP contains an unusual β -lactam structure which significantly alters its molecular recognition properties. The Pirkle 1-J is useful for the direct separation of underivatized β -blocker enantiomers. It can also be used for the separation of the enantiomers of arylpropionic acid NSAIDs, as well as other drugs.

Naphthylleucine (Analytical and Semi-Preparative Columns) phase, a π -electron-donor, is based on N-(1-naphthyl) leucine, covalently bonded to 5 μ m silica through an ester linkage. This phase resolves DNB derivatives of amino acids



as the free acid when used in reversed-phase mode. In the classic normal-phase, this CSP can resolve the amides and esters of DNB amines, alcohols and amino acids.

Davankov ligand exchange chiral stationary phase

DACH-DNB (Analytical to Preparative Columns) CSP was contains the 3,5-dinitrobenzoyl derivative of 1,2-diaminocyclohexane, has been found to resolve a broad range of racemate classes including amides, alcohols, esters, ketones, acids, sulfoxides, phosphine oxides, selenoxides, phosphonates, thiophosphineoxide, phosphineselenide, phosphine-borane, beta-lactams, organometallics, atropisomers and heterocycles.

ULMO (Analytical to Preparative Columns) CSP is based on a 3, 5-dinitrobenzoyl derivative of diphenyl-ethylenediamine. This CSP has a general ability to separate the enantiomers of many racemate classes and is particularly good at separating the enantiomers of aryl carbinols.

Davankov Ligand Exchange Chiral Stationary Phase

The Davankov chiral stationary phase is useful for the separation of underivatized amino acid enantiomers. This phase operates according to the principles of ligand-exchange chromatography (LEC), a technique pioneered by Professor V. Davankov.

The Davankov column requires a mobile phase of aqueous methanol containing copper (II) acetate. Enantioselectivity is extremely high with alphas up to 16 being reported. Regis provides either a Davankov HPLC column, or a kit which allows the user to convert a standard ODS column into a Davankov Chiral Stationary phase.

Both of these Davankov products maintain a stable coating compatible with those mobile phases generally used in amino acid separations.

Davankov Column: A pre-converted Davankov column completes with care and use guide, column test conditions and performance results is available.

Davankov Reagent A Kit: The Davankov Reagent A kit, which contains Davankov Reagent A, a hydroxyproline derivative and copper (II) acetate (sufficient quantities to coat one 15 cm column and prepare mobile phase). The column coating procedure involves dissolving the Davankov Reagent A into methanol/water (80/20) and pumping this mixture through the column. This is followed by a wash with a concentrated solution of $\text{Cu}(\text{OAc})_2$ in methanol/water (15/85). Detailed coating procedures are included with the kit.

Protein-Based Chiral Stationary Phases

Chiral AGP (Micro, Analytical and Semi-Preparative Columns) is the second generation chiral selector based on the α_1 -acid glycoprotein (α_1 -AGP) as the chiral stationary phase. The AGP has been immobilized on spherical, 5 μm particles. The Chiral AGP column is typically used in the reversed-phase mode, where it can be used for the resolution of an extremely broad range of chiral compounds, such as amines, (primary, secondary, tertiary

and quaternary ammonium), acids, esters, sulphoxides, amides, and alcohols. The enantioselectivity and the retention can easily be regulated by the pH of the mobile phase, the buffer concentration and the nature and concentration of the organic modifier²³⁻²⁷.

Chiral CBH (Micro, Analytical and Semi-Preparative Columns) Cellobiohydrolase (CBH) is a stable enzyme which has been immobilized onto 5 μm spherical silica particles. The column is used in reversed-phase mode and is effective for the separation of enantiomers of basic drugs from many compound classes. The retention and the enantioselectivity can be regulated by changes in pH, buffer concentration and the nature and concentration of organic modifier.

Chiral HAS (Analytical and Semi-Preparative Columns) With the Chiral human serum albumin (HSA) column; the enantiomers of many carboxylic acids and amino acids can be resolved directly, without derivatization. Enantioselectivity and retention can be regulated by changing the mobile phase composition, pH, buffer concentration and/or nature of the organic modifier. HSA has been immobilized onto 5 μm spherical silica particles. The surface chemistry of the silica and the method of immobilization provide a stable chiral separation material.

Cyclodextrins chiral stationary phases

Cyclodextrins are macrocyclic molecules containing 6, 7 and 8 glucopyranose units (α -, β -, γ - cyclodextrin respectively) the monomers are arranged so that a shape of a hollow truncated cone is obtained. A relatively hydrophobic chiral cavity is formed, comprised of essentially methylene and 1, 4 glucoside bonds, with which the intercalated solute interacts. In contrast to the interior, the exterior surface is hydrophilic, surrounded by hydroxyls. Mobile phases are usually aqueous solutions mixed with organic solvents; however, normal phase type solvents can also be used. When cyclodextrin stationary phases are used with aqueous mobile phases, the mechanism of retention is based on inclusion complexation. This mechanism represents the attraction of the apolar molecular segment to the apolar cavity. When an aromatic group is present, the orientation in the cavity will be stereoselective due to the interactions with the glucoside oxygens. Linear or acyclic hydrocarbons can occupy positions in the cavity in a random fashion. It is therefore essential that the solute has at least one aromatic ring, if a chiral separation is attempted in the reversed phase mode. The high density of secondary hydroxyls at the larger opening of the torroid is responsible for the preferential hydrogen bonding. Amines and carboxyl groups react strongly with these hydroxyl groups, as a function of the pK of the solute and pH of the aqueous mobile phase. Most productive CYCLOBOND phases CYCLOBOND I 2000, CYCLOBOND I 2000 HP-RSP (highest hit rate) primarily basic chiral compounds have been resolved on the RSP and to a lesser extent both neutrals and acidics. All successful separations have been in the reversed phase mode with the organic component in the range of 5-40%. CYCLOBOND I 2000 DMP (second most



productive CSP in this line) this is a 7i-basic phase. CYCLOBOND I 2000 DNP (new addition) this is an *n*-acidic phase that has demonstrated separations not previously possible on any cyclodextrin phase.

Polysaccharide chiral stationary phases

Several columns now have introduced most widely used polysaccharide-family coated chiral stationary phases for enantioseparations. Many of them prepared with the typical 3, 5-dimethylphenyl-derivatized cellulose and now have added the same derivative for the amylose. These are presented here in no particular order or preference.

CHIRALPAK IA phase as the bonded (immobilized) 3, 5-dimethylphenyl carbamate derivative of amylose. The second in this series of bonded polysaccharide phases, designated CHIRALPAK I B, was introduced as the 3,5-dimethylphenyl carbamate derivative of cellulose. Recently, a new bonded derivative has been introduced designated the CHIRALPAK I C that is described as a 3,5-dichloro-phenyl carbamate derivative of cellulose.

NUCLEOCEL DELTA, the 3,5-dimethylphenyl carbamate derivative of cellulose typically used for normal-phase separations, and NUCLEOCEL DELTA-RP, for reversed-phase use. These phases are coated on silica and offered in 5- and 10- μ m particle sizes for analytical and preparative use.

The amylose designated Kromasil AmyCoat, and cellulose phases designated Kromasil CelluCoat. The silica base is specified as an in-house-developed super-wide pore matrix coated with the typical tris-(3,5-dimethylphenyl carbamate) derivatives of amylose and cellulose, respectively. They are reported to tolerate pressures up to 400 bars. The availability in particle sizes from 3 to 25 μ m offers efficiency and speed for analytical and durability for preparative applications.

Macrocycolic glycopeptides phases

The CHIROBIOTIC, macrocycolic glycopeptides phases, patented products of Advanced Separation Technologies, Inc. (ASTEC, Whippany, New Jersey) have now been incorporated fully into the chromatography section of Supelco (Bellefonte, Pennsylvania) as a result of the purchase of ASTEC in 2006 by Sigma-Aldrich. The product line has been through a vigorous standardized manufacturing procedure at Supelco and is now available around the world through the Sigma-Aldrich distribution network. The CHIROBIOTIC V2 has been shown to have a significant advantage over the CHIROBIOTIC V for chiral amines of all types, especially in the polar ionic mode, The addition of the CYCLOBOND DNP (π -acid) and the CYCLOBOND DMP (π -base) to the Supelco method development protocol also has expanded greatly the selectivity hits for a large number of racemates not separated by other CSPs. An advantage of the Supelco acquisition of Astec has been the availability of other sample preparation technologies like solid-phase extraction (SPE), solid-phase microextraction (SPME), and

a hybrid SPE-molecular imprinted polymer (MIP) to further enhance chiral assay development.

APPLICATION OF CHIRAL DRUG ANALYSIS

Every living body contains amino acids, sugars, proteins, lipid and nucleic acids. All of these are important to living body is of chiral molecule. An interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. When a chemist synthesizes a chiral molecule in an achiral environment using achiral starting materials, an equal mixture of the two possible enantiomers (i.e. a racemic mixture) is produced. In order to make just one enantiomer, some enantioenriched starting material, reagent, catalyst, or template must be present in the reaction medium. Oftentimes, only a single enantiomer of a chiral molecule is desired, as is the case when the target molecule is a chiral drug that will be used in living systems. Drug molecules can be likened to tiny keys that fit into locks in the body and elicit a particular biological response. Since the 'locks' in living organisms are chiral, and exist in only one of the two possible enantiomeric forms, only one enantiomers of the 'key' molecule should be used (the mirror image of our car key will not start our car).

Applications of chiral chromatography has been to the resolution of chiral glycerolipids: One of the important applications of chiral chromatography has been to the resolution of chiral glycerolipids, especially diacylglycerols. Takagi and co-workers were able to resolve a number of chiral glycerol derivatives by preparing the dinitrophenylurethane derivatives for separation on special HPLC columns packed with a silica-based stationary phase to which chiral organic moieties were bound by chemical means. Similar methods have been used for the resolution of prostaglandin derivatives. With the finding that some prostanoids, the isoprostanes, are formed by non-enzymatic methods with no control of stereochemistry, this methodology is especially important. Diastereomeric naphthylethyl and other urethanes especially have been used for the resolution of alcohols and amines of many kinds by column chromatography on non-chiral adsorbents such as silica gel or alumina. Derivatized glycerol compounds, diacyl-*sn*-glycerols especially, with a chiral naphthylethyl isocyanate reagent to form diastereomeric urethane compounds to the resolution of chiral lipids have lead to methods for determining the fatty acid compositions of all three positions of triacyl-*sn*-glycerols (i.e. for stereospecific analysis) by chromatographic means only without any requirement for the use of enzymes.

Role of chiral chromatography in therapeutic drug monitoring and in clinical and forensic toxicology: The ability to unravel complex phenomena associated with drug transport and drug metabolism of chiral drug by chiral chromatographic separations have given dramatically tool to pharmacologists and toxicologists to examine unexpected clinical results involving chiral drugs.



The use of chirality in clinical and forensic situations is illustrated by the relation between the chirality of the drug mefloquine and the intracellular concentrations of the drug cyclosporine is illustrated by examining the effect of the enantiomers of mefloquine on the transport activity of P-glycoprotein (Pgp). These studies were conducted using a liquid chromatographic column containing immobilized Pgp. The results demonstrated that (+)-mefloquine competitively displaced the Pgp substrate cyclosporine whereas (-)-mefloquine had no effect on cyclosporine-Pgp binding. The use of chirality in clinical and forensic situations is also illustrated by the metabolism of the enantiomers of ketamine (KET). The plasma concentrations of (+)-KET and (-)-KET and the norketamine metabolites (+)-NK and (-)-NK were measured in rat plasma using enantioselective gas chromatography. The separations were accomplished using a gas chromatography chiral stationary phase based on β -cyclodextrin. The pharmacokinetic profiles of (+)-, (-)-KET and (+)-, (-)-NK were determined in control and protein-calorie malnourished (PCM) rats to determine the effect of PCM on ketamine metabolism and clearance. The results indicate that PCM produced a significant and stereoselective decrease in KET and NK metabolism. The data suggest that the effects of environmental factors (smoking, alcohol use, diet) and drug interactions (coadministered agents) can be measured using the changes in stereochemical metabolic and pharmacokinetic patterns of KET and similar drugs²⁸⁻³⁰.

Chiral specific applications of essential oils, flavors, and pharmaceutical

Essential oils: Chiral capillary GC has proven to be a convenient method for characterizing essential oils and differentiating natural flavors from those of synthetic origin. Chiral compounds from natural origins usually exist as one predominant optical isomer. Also, the inspection of enantiomeric ratios can characterize regional differences between oils. Although sometimes a result of processing, the presence of racemic pairs (one-to-one ratios of each enantiomer) most often indicates adulteration or unnatural origin. Since most chiral compounds naturally exist as one predominant isomer, resolution is more challenging, especially for components in higher concentrations. For primary constituents in essential oils, select a chiral column that provides a resolution factor value greater than two to overcome possible loss of resolution. Since essential oils are mixtures of many compounds, coelution of peaks and overlapping of certain optical pairs are sometimes hard to avoid. Not all of the chiral compounds found in an essential oil or flavor extract may separate on the same column. Connecting two different columns together is possible, but the elution order of some enantiomers may reverse with this combination, resulting in loss of separation. Dual column analysis is a logical alternative to obtain a more complete enantiomeric profile and to provide confirmational identification of individual constituents. To reduce analysis time, both columns can

be installed into the same injection port for simultaneous confirmation. (Consult Restek's Chro-matography Products Guide for more information about dual column analysis.)³¹⁻³⁴

Flavors: Bergamot oil and a few of the popular fruit flavorings such as raspberry, strawberry, and peach were examined. The compositions of extracts from natural sources were compared to those from commercially available flavored teas and drinks. Some target chiral compounds examined were linalool and linalyl acetate in bergamot oil, oc-ionone and 8-decalactone in raspberry, and γ -lactones in peach extracts.

Drug: Stereochemical properties of chiral drugs have been found in many instances to be the controlling factor concerning activity. One enantiomer may provide a biological function. The other may be inactive or exhibit another functionality, which could result in side effects. In some cases, one optical isomer may be harmful. The FDA requires drug manufacturers to test the individual enantiomers of new drugs for toxicity. Some important examples of compound show chiral natures are given in table.

Table1: Important compound show chiral nature

COMPOUND	COLUMN	MOBILE PHASE
5,5 Hydantoin	All Chirobiotics	MeOH
Oxazepam	V/T/R	MeOH
*N-Amine	V/V2	100/0. Iw%,MeOH/ATFA
*N-Amine	V	30/70,MeOH/TEAA,4.1
Methodone	V2	100/0. Iw% MeOH/ATFA
Methodone	HP-RSP	20/80, ACN/NH40Ac,3.6
*Propranolol	T/T2	100/0. Iw% MeOH/ATFA
*Propranolol	TAG	100/0. Iw% MeOH/ATFA
Terbutaline	T/T2/TAG/V	100/0. Iw% MeOH/ATFA
Metoprolol	T	100/0. Iw%,MeOH/ATFA
*Bendroflumethiazide	V	10/90,THF/NH4 NO3
*Bendroflumethiazide	T	30/70, MeOH/H2O
Naproxen	V	10/90, THF/NaCitrates
Naproxen	R	20/80, MeOH/TEAA,5.5
Ibuprofen	V	10/90, THF/NaCitrates
Albuterol	V/T	100/0. Iw%,MeOH/ATFA
Mianserin	V	100/0. Iw%,MeOH/ATFA
Mianserin	T	100/0. Iw%,MeOH/ATFA
Mandelic acid	T/R	30/70, MeOH/pH 4.5
Mandelic acid	T/R	100/0. Iw%,MeOH/ATFA
Bupvacaine	V/V2	100/0. Iw%,MeOH/ATFA
Nicardipine	V/V2/T	100/0. Iw%,MeOH/ATFA
Ritalin	T2	100/0. Iw%,MeOH/ATFA
Omeprazole	R	40/60, EtOH/Heptane
Omeprazole	R	30/70, MeOH/HOAc,4.1
Amphetamine	V2	100/0.05w%,MeOH/ATFA
Methamphetamine	V2	100/0.05w%,MeOH/ATFA
Dextro/Levorphanol	V/V2	100/0. Iw%,MeOH/ATFA
Dextro/Levo methorphan	V/V2	100/0. Iw%,MeOH/ATFA
Butorphanol	T/T2	100/0. Iw%,MeOH/ATFA
Ketoprofen	R	100/0.02w%,MeOH/HOAc
a-Me a-Ph succinimide	R	20/80,EtOH/Hex
a-Me a-Ph succinimide	TAG/T/V	20/80,EtOH/Hex



CONCLUSION

Chiral analysis is an essential undertaking throughout Drug Discovery and Development since the enantiomeric forms of a chiral drug substance often possess completely different pharmacological effects with one enantiomer producing a potent, desired effect and the other being inactive or even toxic.

REFERENCES

1. Enquist M., Hermansson J., *Chirality*. 1, 209-215 (1989).
2. Walhagen A., Edholm L-E., Kennedy B-M., L.C. Xiao, *Chirality*. 1, 20-26, (1989)
3. Ward T.J., Armstrong D.W., *Chromatographic Chiral Separations* (M. Zief, L.J.Crane, eds.) pp. 131-163, Marcel Dekker, New York, (1988).
4. Allenmark S.G., *Chromatographic Enantioseparation*. pp. 75-89, Ellis Horwood, Chichester, (1988).
5. Lindner W., *Chromatographic Chiral Separations*. (M. Zief, L.J. Crane, eds.), pp. 91-130, Marcel Dekker, New York, (1988).
6. Lough W.J., *Chiral Liquid Chromatography*, Blackie, Glasgow (1989).
7. Ahuja S., *Chiral Separations by Liquid Chromatography*, American Chemical Society, Washington, DC (1991).
8. Wainer I.W., Alembik M.C., *Chromatographic Chiral Separations* (M. Zief, L.J. Crane, eds), pp. 355-384 (1988).
9. Wainer I.W., *Drug Stereochemistry. Analytical Methods and Pharmacology*, Marcel Dekker, New York (1993)
10. Gorog S., *Enantiomeric Derivatization, in Detection-Oriented Derivatization Techniques in Liquid Chromatography* (H. Lingeman and W.J.M. Underberg, Eds.), pp 193-216, New York, Marcel Dekker (1990).
11. Gorog S., *Enantiomeric derivatization, in Encyclopedia of Separation Science* (I.D Wilson, T.R. Alard, C.F. Poole and M. Cook, Eds.), Academic Press, London (2000).
12. Skidmore M.W., *Derivatization for chromatographic resolution of optically active compounds, in Handbook of Derivatives for Chromatography*, (K. Blau and J. Halket, Eds.), pp 215-252, Wiley, Chichester (1993).
13. Souter R.W., *Chromatographic Separation of Stereoisomers*, CRC Press, Boca Raton, FL (1985).
14. Stevenson T. T and Wilson I.D., *Chiral Separations*, Plenum Press, New York (1989).
15. Zief M. and Crane L.J. *Chromatographic Chiral Separations*, Marcel Dekker, New York (1987).
16. Lim H.K., Hubbard J.W. and Midha K.K., *Enantioselective gas chromatographic assays with electron-capture detection for methoxyphenamine and its three primary metabolites in human urine* *J. Chromatogr.* 378, 109-123 (1986).
17. Ladanyi L., Sztruhar I., Slegel P. and Vereczkey-Donath G., *Direct and indirect high-performance liquid chromatography enantioseparation of trans-4-hydroxy-2-nonenic acid* *Chromatographia* 24, 477-481 (1987)
18. Slegel P., Vereczkey-Donath G., Ladanyi L. and Tofh-Lauritz M., *J. Pharm. Biomed. Anal.* 5, 665-673 (1987)
19. Demian I. and Grisphover D.V., *Derivatives for HPLC Analysis* *J. Chromatogr.* 466, 415-420 (1989)
20. Toyo'oka T. and Liu Y.M., *Analyst* 120, 385-390 (1995)
21. Matuszewski B.K., and Kiganda M., *Analytical chiral separation of the stereoisomers of a novel carbonic anhydrase inhibitor* *Pharm. Res.* 11, 149-154 (1994).
22. Rudd-Christensen M., Aasen A.J., Rasmussen K.E., Salvesen B., *High-performance liquid chromatographic determination of (R)- and (S)-propranolol in human plasma*, *J. Chromatogr.* 491, 355-366 (1989).
23. Sedman J., Gal J., *Enantioselective drug monitoring of (R)- and (S)-propranolol in human plasma via derivatization with optically active (R,R)-O,O-diacetyl tartaric acid anhydride* *J. Chromatogr.* 300, 155-164 (1984).
24. Schaefer H.G., Spahn H., Lopez L.M., *Simultaneous determination of propranolol and 4-hydroxypropranolol enantiomers after chiral derivatization using reversed-phase high-performance liquid chromatography*, *J. Chromatogr.* 521, 351-359, (1990).
25. Dalglish C.E., *J. Chem. Soc.* 3940-3942 (1952).
26. Finn J.M., *Chromatographic Chiral Separations*. (M. Zief, L.J. Crane, eds), p. 53-90, Marcel Dekker, New York, (1988).
27. Wainer I.W., Doyle T.D., *Analytical Criteria for Chiral High-Pressure Liquid Chromatography*, *J. Chromatogr.* 306, 405-411 (1984).
28. Drayer D.E., *Drug Stereochemistry. Analytical Methods and Pharmacology*.
29. Gal J., *Drug Stereochemistry. Analytical Methods and Pharmacology*, pp. 77-112, Marcel Dekker, New York (1988).
30. Fitzgerald R.L., Ramos J.M., *Resolution of methamphetamine stereoisomers in urine drug testing: urinary excretion of R(-)-methamphetamine following use of nasal inhalers*, *J. Anal. Toxicol.* 12, 255-259 (1988).
31. Allenmark S.G., *Chromatographic Enantioseparation:*



- Methods and Application, Ellis Horwood, Chichester (1989).
32. Allenmark S., Chiral Separations by HPLC. Applications to Pharmaceutical Compounds. (A.M.Krstulovic, ed), pp. 285-315, Ellis Horwood, Chinchester, (1989)
33. Chu Y-Q, Wainer I.W., The application of achiral/chiral coupled-column high-performance liquid chromatography to biomedical analysis Pharm. Res. . 5, 680-683 (1988).
34. Wainer I.W., Stiffln R.M., Wainer I.W., Stiffln R.M., Chiral supercritical fluid chromatography of phenylpropanols and related compounds, J. Chromatogr.424. 158-162 (1988).

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