Pure & Appl. Chem., Vol. 63, No. 8, pp. 1119–1122, 1991.
Printed in Great Britain.

ADONIS 2069222091001012

Resolution of chiral drugs

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Abstract - Many drugs are administered as racemates, yet the enantiomers may have important pharmacodynamic and/or pharmacokinetic differences. Chiral analytical techniques are necessary to properly assess and understand such differences. Novel techniques to separate drug enantiomers present in serum and/or urine have recently emerged. This review describes commonly used approaches to chiral drug analysis in: diastereomeric derivatization, chiral mobile-phase additives, chiral stationary phases, and enantioselective immunoassays. Each of these techniques has advantages and disadvantages and no single approach has emerged as the method of choice.

INTRODUCTION

Pharmacokinetic and pharmacodynamic differences between the enantiomers of drugs administered as racemates are increasingly appreciated (ref.1-3). Thus, analytical methods for the chiral resolution of such drugs are paramount to a full understanding of enantioselective drug action and disposition. Since enantiomers have identical chemical and physical properties in a symmetrical environment, conventional chromatographic techniques can not achieve chiral resolution.

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 physiochemical 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.

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:

Drug		CDR				
(R + S)	+	R′	⟶	R - R′	+	S - R′

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:

Drug	CDR		
(R + S)	R′ + (S′)	⟶	R-R′+ S-R′+ R-S′+ S-S′

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 (ref.4). Precautions must also be taken to avoid the possibility of "kinetic resolution" of drug enantiomers resulting from their differential reaction rates with the CDR (ref.4).

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 (ref.4,5).

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 (ref.6), and the use of (S)-α-methoxy-α-trifluoromethylphenylacetyl chloride for the GC chiral analysis of tocainide enantiomers in plasma (ref.7). Applications employing achiral HPLC columns include the analysis of propranolol and 4-hydroxypropranol enantiomers in serum after derivatization with (+)-(R)-phenylethylisocyanate (ref.8), and the use of (-) -camphanoyl chloride as a CDR for the chiral analysis of racemic proxyphylline in plasma (ref.9).

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 (ref.10). 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 requiste spectral or fluorescent properties to the molecule.

Although much progress has been made in elucidating chiral recognition mechanisms, this knowledge in many cases is 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 (ref.11,12).

Chiral stationary phases

Pirkle-type CSPs. These CSPs, developed by Dr. W.H. Pirkle, were designed for HPLC use and are based on the ionic or covalent attachment of one enantiomer of an amino acid derivative [eg. (R)-N-(3,5-dinitrobenzoyl)phenylglycine] to aminopropyl silica (ref.13). Transient diastereomeric complexes involve electron donor-acceptor (π - π) interactions, hydrogen bonding and dipole-dipole interactions (ref.13).

A number of Pirkle-type CSPs are commercially available (ref.14). They are used most often in the normal phase mode. Considerable progress has been made in elucidating the mechanisms involved in chiral discrimination with Pirkle-type CSPs (ref.13), thus allowing the rationale selection of a particular column to achieve enantioresolution of chiral drugs. However, there have been only limited applications of Pirkle-type CSPs for the analysis of chiral drugs in biological fluids. One example is the use of the ionic form of a (R)-N-(3,5 dinitrobenzoyl)phenylglycine CSP to resolve the enantiomers of propranolol after extraction from serum (ref.15).

Hydrogen-bonding CSPs for GLC. The development of CSPs for gas chromatography has been hampered by problems of thermal stereochemical instability of these phases. One commercially available chiral capillary GC column (Chirasil-Val) contains L-valine-*tert*-butylamide as the chiral descriminator covalently attached to a polysiloxane matrix (ref.16). Chiral resolution presumably occurs as the result of the formation of hydrogen-bonded diastereomeric association complexes with the CSP. An interesting example of the application of Chirasil-Val is the separation of the enantiomers of mephenytoin and its demethylated metabolite present in serum following administration of the racemate. Using this technique, stereochemical differences in the metabolism and elimination of mephenytoin enantiomers was demonstrated (ref.17).

Cyclodextrin CSPs. Commercially available cyclodextrins are cyclic oligomers of six, seven or eight glucose units designated α,β and γ-cyclodextrin, respectively. These cyclodextrins are chiral (each glucose unit contains five chiral centers) molecules that resemble a truncated cone. The interior surface of the cone forms a relatively hydrophobic chiral cavity rimmed by the secondary 2 - and 3 - hydroxyl groups at the larger opening and by the primary 6-hydroxyl groups at the smaller orifice (ref. 18).

Cyclodextrins can form inclusion complexes with molecules of the appropriate size and spatial configuration. The stability of these complexes depends on the goodness of fit of a relatively nonpolar (preferably aromatic) side chain of the analyte and the hydrophobic cavity of the cyclodextrin. Hydrogen bonding with the hydroxyl groups at the cavity entrance contribute to inclusion complex stability and to enantioselectivity (ref.18).

The chiral cavity of β-cyclodextrin is of appropriate size for the formation of inclusion complexes with a number of drugs (ref.18). Covalent attachment of cyclodextrin via a spacer arm to silica gel results in a CSP suitable for reverse phase HPLC use (ref.18). The enantiomers of ibuprofen were analyzed using a β-cyclodextrin CSP (Cyclobond I), after their extraction from serum, urine or bile (ref.19). For the analysis of terbutaline enantiomers in serum, an achiral-chiral coupled column method was required in which chiral resolution was accomplished using a β-cyclodextrin CSP (ref.11).

Protein-bound CSPs. Proteins, because they consist of L-amino acids and posses an ordered three-dimensional structure, may bind drugs in a stereoselective manner (ref.20). This phenomena has been capitalized upon to prepare silica-bound protein CSPs for reverse phase chiral chromatography. Albumin (Resolvosil), α_1-acid glycoprotein (EnantioPac; Chiral-AGP) and ovomucoid (Ultron ES-OVM) CSPs are commercially available.

Separation mechanisms for protein CSPs, albeit not well understood, probably involve hydrophobic, hydrogen bonding and electrostatic interactions (ref.20). These CSPs can resolve a wide variety of chiral drugs although with relatively low efficiency (ref. 20).

A second-generation α_1-acid glycoprotein CSP with much improved efficiency (Chiral-AGP) has been used for analysis of atenolol enantiomers in plasma and urine (ref.21), for the resolution of alfuzosin enantiomers in plasma (ref.22), and for a pharmacokinetic study of metoprolol enantiomers after administration of the racemate (ref.23). To avoid interferences by endogenous substances and/or metabolites, it may be necessary to couple the CSP in series with an achiral column. Such a coupled-column approach was required for chiral analysis of disopyramide (ref.24), and for verapamil and its metabolite, norverapamil (ref.12). In each case, enantioresolution was accomplished with Chiral-AGP. Likewise, a coupled column technique was necessary for chiral analysis of warfarin (ref.25) and leucovorin (ref.26) enantiomers in serum using Resolvosil for the CSP.

Cellulose-derived CSPs. Cellulose and its derivatives have a certain degree of rigidity and assume extended helical conformations. Presumably a chiral cavity or space exists on or within these derivatives which accounts for their chiral recognition properties (ref.27).

Cellulose ester and carbamate derivatives (such as cellulose triacetate, tris (3,5-dimethylphenyl)carbamate) have been coated on silica gel and are commercially available (ref. 27). Chiral recognition mechanisms are not well understood but apparently involve hydrogen-bonding and dipole-dipole interactions between the analyte molecule and the ester or carbamate linkages of the CSP. These cellulose-derived CSPs are used in the normal phase mode typically with hexane containing an alcohol modifier as the mobile phase.

These CSPs have been used to achieve enantioresolution of chiral drugs after extraction from serum. Specific applications include the direct chiral analysis of β-adrenergic blockers such as metoprolol (ref.28) and celiprolol (ref.29). Other applications include chiral analysis of disopyramide and its metabolite (ref.30), the calcium channel blocker, felodipine (ref.31), and the anticovulsant drug, ethotoin (ref.32).

Chiral mobile phase modifiers

Chiral selectors may be added to the mobile phase. Eantioresolution using conventional HPLC columns occurs as a result of the differential transient diastereomer complex stability and/or differential interactions of these complexes with the stationary phase (ref.33,34). Examples of chiral mobile phase modifiers include cyclodextrins (ref.34), and ion-pairing agents such as N-benzoxycarbonylglycyl-L-proline (ref.33). The latter chiral complexing agent was used in a mobile phase for the separation of propranolol enantiomers following their extraction from plasma (ref.35).

Enantioselective immunoassays

The binding of chiral ligands to antibody binding sites may be stereoselective. Thus, if an animal is immunized with an optically pure hapten, the antibodies formed may demonstrate very low affinity for the hapten antipode (low cross reactivity) (ref.36). Stereoselective radioimmunoassays for (-)-ephedrine and (+)-ephedrine (< 2% cross-reactivity) (ref.37), for (R)-and (S)-pentobarbital (< 1.5% cross-reactivity) (ref.38), and for (R)-and (S)-warfarin (ref.39) were employed to demonstrate enantioselective disposition of these drugs following administration of the respective racemates. Commercially available stereoselective immunoassays are available for the detection of (+)-(S)-methamphetamine (ref.40,41). In forensic urine drug screening, it is important to distinguish between (+)-(S)-methamphetamine (schedule II drug) and (-)-(R)-methamphetamine which may be present in over-the-counter nasal inhalers (ref. 6). The latter may also arise from metabolism of selegiline, prescribed as an antiparkinson drug (ref.42).

Although immunoassays may be highly enantioselective, they are not immune to interference by drug metabolites, closely related analogues or by endogenous substances in biological fluids (ref.36). The preparation of enantioselective antibodies, especially by hybridoma techniques, is clearly beyond the realm of capability of many laboratories. Once prepared and validated, such antibodies provide the means for rapid analysis of large numbers of samples by automated non-isotopic immunoassays.

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