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REVIEW

DIRECT SEPARATION OF DRUG ENANTIOMERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH CHIRAL STATIONARY PHASES

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1. INTRODUCTION

A significant proportion (approximately 25%) of the most widely prescribed drugs are sold as racemic mixtures. The individual isomers of such mixtures frequently differ in pharmacological or metabolic activities [1], and often only one isomer is therapeutically active. For example, (*S*)-warfarin is more potent anticoagulant than the (*R*)-isomer, but is eliminated more rapidly from plasma. Moreover, other drugs have been shown to interact differently with warfarin enantiomers, for instance, when coadministered with phenylbutazone, the hepatic clearance of the (*S*)-isomer of warfarin is significantly decreased, whereas that of (*R*)-isomer is increased compared with administration of warfarin alone [2,3].

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Stereoselective differences in potencies have also been observed with enantiomers of propranolol, the (*S*)-isomer being 100 times more potent as a β-blocker and having a longer plasma half-life than the (*R*)-form [4]. These are just few examples out of many where enantiomers of a chiral drug possess different pharmacological characteristics.

Except for their interaction with plane-polarised light enantiomers have identical physical and chemical properties in a symmetrical environment. However, if they are introduced in a chiral environment such as human body they often act as two different molecules with different biological activities. The differences in biological activities of the two optical isomers of a given drug may be due to several reasons, for example, isomers may interact differently with optically active components of the living body (receptors, proteins, enzymes) leading to differences in absorption, distribution and metabolism. When seeking a correlation between drug levels in blood and clinical efficacy, it is important to be able to separate and determine optical isomers in biological fluids. Since their physicochemical properties are similar, the separation and quantification of optical isomers have been traditionally regarded as difficult and time-consuming. However, this situation has changed, and more research work has been carried out lately in devising efficient ways of separating optical isomers particularly using high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs). It would seem appropriate, therefore, to review some of the more recent developments that have been made in this area with particular reference to enantiomeric drug analysis.

2. INDIRECT METHODS

A variety of approaches for the chromatographic separation of enantiomeric mixtures have been reported [5,6]. Among these, indirect methods are based on the reaction of racemic mixture with a chiral reagent to form a pair of diastereomers. The diastereomers possess different physicochemical properties and can generally be separated by gas chromatography (GC) or HPLC on a non-chiral column. Among the derivatising agents N-trifluoroacetyl-1-propyl chloride is the most widely used for both GC and HPLC [7].

Although the indirect methods have been used extensively in the past, e.g. in the analysis of enantiomers of β-blockers [7], they have certain disadvantages. (1) They are time-consuming, tedious, and require optically pure (and hence expensive) derivatising agents, since the enantiomeric contamination of the reagents could lead to false determinations. (2) They often fail to accomplish total separation of enantiomers. (3) As the resulting diastereomers have different physicochemical properties, the rate of formation may not be the same for each member of the pair. This may result in the generation of two diastereomeric products differing in proportions from the starting enantiomers. (4) A further chemical treatment is necessary if the starting enantiomers are to be reclaimed.

3. DIRECT METHODS

In recent years, as alternative procedures to indirect methods, methods for the direct resolution of enantiomers without prior derivatisation have come into use. These methods involve the use of CSPs that form transient diastereomeric complexes with solute enantiomers. The difference in stability between the complexes leads to a difference in retention time and hence separation. These methods avoid some of the drawbacks of the indirect methods mentioned earlier, and are relatively simple and less time-consuming. CSPs can be used in both GC and HPLC, but HPLC has the advantage over GC [6,8], since the enantiomers need not be volatile and, if necessary, can be recovered with relative ease. Furthermore, GC requires more extensive sample clean-up procedures and the column temperature can reduce the stability difference between enantiomers resulting in incomplete separation. The column temperature may also cause the racemisation of the solute during analysis. Due to these reasons, HPLC is presently the most widely used technique for enantiomeric separations.

In HPLC, instead of CSPs, chiral mobile phase additives such as camphorsulphonic acid [9] or quinine [10] can also be used, but this approach has some disadvantages, e.g. many additives are costly, are not commercially available, and their mode of operation is complex. Moreover, for preparative applications the chiral additive must be removed from the enantiomeric solutes. For these reasons the present trend is towards the use of CSPs whenever HPLC is considered for enantiomeric drug analysis [11,12]. Recently a number of CSPs have been described in the literature which have shown to be efficient for the separation of enantiomeric compounds including drugs. Of these, many are now commercially available. Däppen et al. [13] have discussed in detail the applications and limitations of commercially available CSPs for HPLC. Also included in their article are the structural formulae of the stationary phases and the list and full addresses of the suppliers of the CSPs.

3.1. Dinitrobenzoyl phases

The first commercially successful CSP was developed by Pirkle et al. [14]. It consists of N-(3,5-dinitrobenzoyl)phenylglycine ionically or covalently linked to aminopropyl silica. Since then several dinitrobenzoyl (DNB) phases (e.g., DNB derivatives of leucine) have been synthesized and are commercially available. These CSPs are designed on the basis of the three-point recognition model proposed by Dalgliesh [15], who postulated that chiral recognition requires at least three simultaneous interactions between the CSP and the solute. A DNB-type stationary phase has a number of possible sites available for different kinds of interactions with the solute molecule which are believed to be responsible for chiral recognition. Resolution of racemic mixtures occur through a combination of π–π, hydrogen bonding, dipolar or steric interactions. This enables this stationary phase to be used for the separation of a large number of compounds [16]. However, despite its popularity in other areas, the DNB stationary phase has limited applications in the drug analysis field. This is because the enantioselectivity and stability of this phase are generally much greater under normal-phase

conditions whereas HPLC drug analysis is usually performed under reversed-phase (RP) conditions. In spite of this, use is made of DNB columns in drug analysis, for example, in separating enantiomers of diazepam analogues [17] and of primaquine and its metabolites [18].

Yang et al. [19] have described the separation of enantiomeric barbiturates, succinimides, and related compounds on four commercially available Pirkle type CSPs, i.e. the ionically or covalently bonded DNB-phenylglycine and DNB-leucine phases. The resolution factor and other chromatographic parameters for the test compounds varied from one stationary phase to another, but in the majority of the cases, the leucine-based CSPs demonstrated a higher enantiomeric selectivity and efficiency than the phenylglycine-based CSPs. Sometimes it is necessary to derivatise the drug in order to obtain good separation on the DNB column. Wainer et al. [20] have separated cyclic 2-oxazolidine derivatives of propranolol enantiomers on a DNB-phenylglycine column and detected by fluorescence detector. The method was applied in the determination of the enantiomeric composition of propranolol in serum and was shown to be suitable for pharmacokinetic studies.

Other more recently developed normal-phase media for enantiomeric separations include Supelco's phenylethylurea phase [Supelcosil LC-(*R*)-Urea] and those based on derivatives of readily available chiral compounds such as tartaric acid [21,22], glucose [23] and quinine [24,25]. These phases seem promising but need further evaluation before gaining acceptance in the drug analysis laboratory.

3.2. Protein bonded phases

The stereoselective interactions of proteins with solutes have been used for the resolution of enantiomers. Proteins such as albumin can undergo stereoselective interactions with a large number of pharmacologically active compounds, hence protein-based CSPs appear to have a wide applicability in the field of drug bioanalysis. Because of their complex structure, however, the mechanism of chiral recognition by proteins is largely unknown. The retention seems to be mainly based on hydrophobic and electrostatic (coulombic) interactions, although hydrogen bonding and charge transfer interactions may also be of significance [26].

Two CSPs utilizing the interactions between enantiomers and proteins are commercially available. One is based on plasma protein α_1-acid glycoprotein (α_1-AGP) immobilized on silica gel, and the other consists of bovine serum albumin (BSA) covalently bonded to silica gel. The BSA column is particularly useful for the separation of enantiomers of amino acids and their derivatives [26]. The α_1-AGP column, on the other hand, exhibits high enantioselectivity for a number of acidic [27] and basic [28] drugs and hence is widely used for the separation of drug enantiomers.

Both protein-based columns are used with buffered aqueous mobile phases with low concentration of organic modifiers such as 1- or 2-propanol or N,N-dimethyloctylamine (DMOA). The aqueous mobile phase allows direct injection of aqueous samples onto the column, which facilitates analysis of biological samples. It is

possible to optimize a separation by a proper selection of mobile phase parameters, such as temperature, pH, ionic concentration, and the concentration of organic modifier, since the retention properties of protein columns are highly dependent upon these parameters. Protein bonded phases have a high selectivity but a low capacity as the load of protein on the column is low. Therefore, care should be taken not to overload the column especially when the optimal separation is required. It is also advisable to use a pre-column or a column-switching technique while dealing with biological samples.

Hermansson and Eriksson [27] have made a detailed study of the stability of the α_1-AGP column and found that it can be used at elevated temperatures, and is very stable in the presence of a water–propanol mixture or pure propanol. However, like other RP columns, amine additives such as DMOA in the mobile phase in combination with a high pH decrease the life-time of the α_1-AGP column.

3.2.1. Some applications of the α_1-acid glycoprotein column. The optical isomers of several drugs from different families (e.g., anti-inflammatory analgesics) were resolved using an α_1-AGP column and UV detection [27,29,30]. Separations were performed using a mixture of aqueous buffer and organic modifier (2-propanol or DMOA). Retention of the isomers was adjusted by selection of temperature (15 or 35°C), pH (3–7.5), ionic strength of the electrolyte (sodium chloride), concentration of organic modifier in the mobile phase and flow-rate. It was found that an increase in protein loading resulted in an increase in capacity factors. Enantiomers of most drugs were resolved directly but some drugs (notably β-blockers) needed conversion to their cyclic 2-oxazolidines prior to separation [29,31]. A similar study on α_1-AGP column was conducted by Schill et al. [28] with particular emphasis on the separation of optical isomers of cationic drugs such as cocaine and methadone. Using this column it was possible to achieve better than 95% resolution of a majority of racemates.

The effect of various factors (concentration of 2-propanol and sodium chloride in the mobile phase, and buffer pH) on chiral resolution of nomifensine on α_1-AGP column was studied by Noctor and co-workers [32,33]. The separation was found to be very sensitive to changes in the above factors. Flow-rate and temperature also exerted some effects. For nomifensine, the optimal concentration of 2-propanol was 3.5% (v/v) in 8 m*M* phosphate buffer; sodium chloride concentration, 0.1 *M*; and pH of buffer was 7.0. The separation time was 33 min and the wavelength of detection was 215 nm.

An enantioselective HPLC method was developed using a chiral α_1-AGP column for measuring chloroquine and desethylchloroquine in plasma and urine followed by oral administration of racemic chloroquine in volunteers [34]. Although the protein column separates chloroquine and desethylchloroquine enantiomers, it cannot separate chloroquine from desethylchloroquine. It was therefore necessary to isolate these two compounds first by using a conventional HPLC procedure under normal-phase conditions. The separated chloroquine and desethylchloroquine fractions were collected and further analysed using an α_1-AGP column and a fluorescence detector. The sensitivity of the method was sufficient to allow analysis within the concentration range encountered in pharmacokinetic studies. In certain individuals metabolism and renal excretion of the drug were found to be stereoselective.

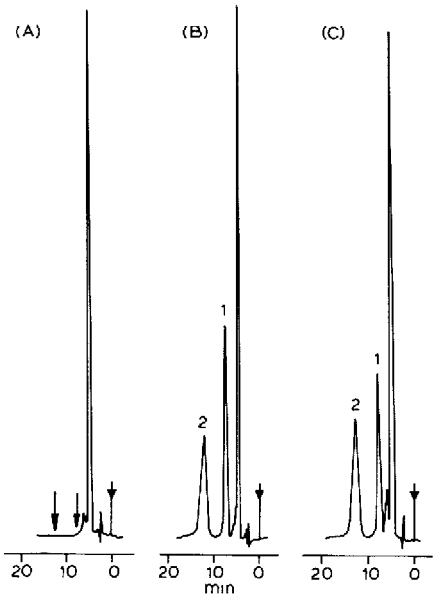

Fig. 1. Separation of (*R*)- and (*S*)-disopyramide isolated from human plasma. (A) Blank plasma, (B) plasma spiked with racemic disopyramide (1.5 μg/ml); (C) plasma sample obtained from a patient. Peaks: 1 = (*R*)-disopyramide; 2 = (*S*)-disopyramide. Reproduced from ref. 35 with permission

The direct resolution and quantification of (*R*)- and (*S*)-disopyramide isolated from human plasma was accompanied by using a combination of a conventional RP column (used as a pre-column) and the α_1-AGP column [35] (Fig. 1). Disopyramide and its metabolite monodesisospropyldisopyramide were first separated on an RP pre-column followed by the resolution of enantiomers on an α_1-AGP column and UV detection. According to the authors the presence of pre-column was necessary, because without it, the (*S*)-metabolite was eluted approximately at the same time as the (*R*)-drug despite the fact that many mobile phase combinations were tested. If the RP column was coupled before the protein column, complete separation of the enantiomers of disopyramide and its metabolite was achieved. The plasma levels of the (*R*)-drug were about half of those of the (*S*)-isomer, though the half-lives of both enantiomers were similar.

3.3. Cyclodextrin bonded phases

A relatively new approach to chiral recognition involves inclusion of the analyte into a chiral cavity of a cyclodextrin (CD) molecule [36–38]. CDs are cyclic oligomers in which glucose units are joined together to form a torroidal structure with a relatively hydrophobic centre and a polar outer surface. A variety of water-soluble and -insoluble compounds can fit into the hydrophobic cavity of the CD molecule, thereby forming reversible inclusion complexes of different stability. If the less polar (preferably aromatic) part of the solute molecule fits exactly into the CD cavity, and the substituents interact with chiral exterior (polar hydroxy groups of the glucose units), enantiomeric separation can be expected. The better

the fit of the guest molecule, the better the separation. Small molecules that are completely enclosed by the CD cannot be separated. Those molecules that form weaker complexes frequently retain sufficiently long to give resolution. If the molecules are too large compared to the CD cavity, there may be little or no interaction, unless certain groups or side chains of the molecules can enter the cavity effectively. An outer rim of the hydroxyl groups in the CD molecule can be derivatised, for example, acetylated. This changes the selectivity of the CD phase presumably by enhancing the hydrogen bonding interactions between the rim of the CD molecule and the solute.

A CD packing consists of CD molecules covalently bound to silica gel to form a hydrolytically stable bonded phase. The three smallest homologues of CD, i.e. α, β, and γ forms, which contain 6, 7 and 8 glucose units, respectively, are available commercially. The size of the hydrophobic cavity increases with the increase in the number of glucose units, allowing a wider range of compounds to be separated. One member of the series is an acetylated form of β-CD.

CDs are the first commercial CSPs intended to be used in a traditional RP mode. Typical eluents are those containing methanol or acetonitrile in water. Retention can be increased by increasing the water content and the separation of the ionisable compounds can be affected by altering the pH of the mobile phase. Despite the experimental similarities to traditional RP-HPLC, there are a number of significant differences, e.g., retention is predominantly due to inclusion complex formation. The elution order of most compounds in the RP mode on CD media can be different from that using traditional RP columns. This suggests that the retention mechanisms are not the same [37]. Furthermore, temperature, ionic strength, and pH effects are generally more pronounced on CD bonded phases than on RP columns. CD columns can also be used in a normal- or a mixed-phase mode but the separation mechanism is generally different, i.e., not entirely due to the formation of inclusion compounds.

The aqueous compatibility of CD and its unique molecular structure indicate the possibility of wider applications of CD bonded phase in the drug analysis area than other CSPs. The number of compounds resolved by this stationary phase is encouraging which is evident from the pioneering work of Armstrong and coworkers [39,40]. Enantiomers of propranolol, chlorthalidone, ketoprofen and many more drugs have been separated; however, enantiomers of certain drugs such as doxylamine and warfarin were not effectively resolved, presumably due to the absence of differential interaction at the mouth of the CD cavity [40].

It is still early days for CD stationary phases but since they are commercially available [8,13], the next few years should see their use in chiral HPLC of drugs. McClanahan and Maguire [41] have separated the enantiomers of urinary metabolite of phenytoin on a β-CD column. The metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin was first isolated from urine by a conventional RP (C_{18}) column. This was followed by enantiomeric separation on a β-CD column with UV detection. This method can be used to study the stereroselective pathways in phenytoin metabolism. Recently, in order to characterise the enantiodependent disposition of hexobarbital in rat, Huang Chandler et al. [42] have separated *d*- and *l*-hexobarbital in rat blood by HPLC with a β-CD column (Fig. 2). Marked

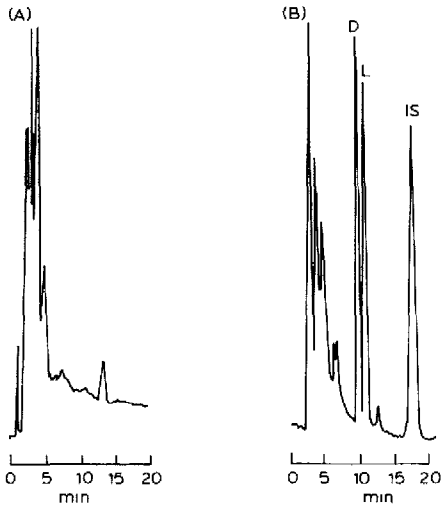

Fig. 2. Chromatograms of a blank blood sample (A) and a blood sample spiked with 1 0 μg of racemic hexobarbital and 0.42 μg of alphenal internal standard (B) Peaks: D = *d*-hexobarbital; L = *l*-hexobarbital; IS = alphenal. Reproduced from ref. 42 with permission.

differences in the disposition (half-life and clearance) of the enantiomers were observed, indicating more rapid metabolism of the *d*-isomer. β-CD CSP has also found use in achiral separations, for example, in the separation of antihistamines [43] and of tamoxifen geometric isomers and metabolites [44].

3.4. Synthetic polymer phases

Several different cellulose derivatives (e.g., esters, ethers, carbamates) have been proposed as CSPs in HPLC. They can be used for separations involving non-polar and polar interactions and hydrogen bond formation. Both polar (methanol or ethanol in water) and non-polar (hexane–2-propanol) solvents are used as mobile phases with these CSPs. Blaschke [45] has resolved a large number of chiral drugs, such as benzodiazepines, by chromatography on optically active microcrystalline cellulose triacetate. The chromatographic resolution is supposed to be due to the inclusion of an enantiomer into chiral cavities of the polymeric network [45]. Okamoto and co-workers [46,47] have tested several silica-based cellulose triphenylcarbamate derivatives as CSPs for optical resolution of β-blockers, and alkaloids and other compounds with mobile phase of 0.1% diethylamine in hexane–2-propanol (4:1) and use of a polarimetric detector. The main chiral recognition sites are considered to be the polar carbamate groups, which can interact with a solute via hydrogen bonding or dipole–dipole interactions. These interactions may further be influenced by the nature of the substituents on the phenyl groups. Cellulose tris(3,5-xylylcarbamate) and cellulose tris(3,5-dimethylphenylcarbamate) stationary phases were found most suitable for optical resolution of β-blockers and alkaloids, respectively, providing optimum separation.

Other CSPs in polymer series consist of optically active poly(triphenylmethyl

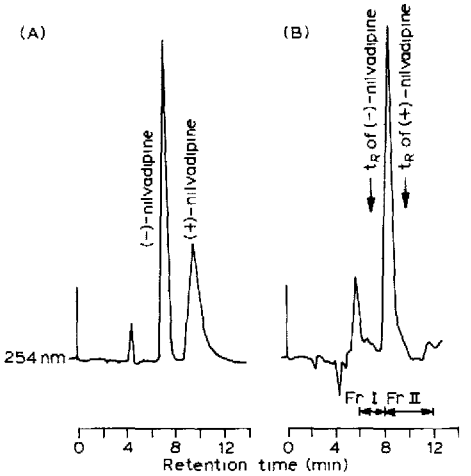

Fig. 3. Chromatograms of (A) racemic nilvadipine and (B) extract of human plasma containing (+)-nilvadipine (1 ng/ml) and (−)-nilvadipine (0.5 ng/ml). Reproduced from ref. 49 with permission of the American Pharmaceutical Association.

methacrylate) (PTMA) or its pyridyl derivative coated silica gel. They are unique in that their chirality arises purely from their rigid helical structure [48]. Enantiomers are retained differently if their attachment between the layers of the helix is different and therefore a chiral separation is feasible. Methanol–water or hexane–2-propanol can be used as mobile phases with polymethacrylate stationary phases. As these phases are prone to irreversible adsorption, biological samples should not be injected without a pre-column [13]. Enantiomers of nilvadipine in human plasma have been separated on PTMA with methanol–water (95:5) mobile phase, 0.8 ml/min flow-rate and detection at 254 nm [49] (Fig. 3). The effluents containing the respective isomers were collected and analysed further by GC–MS. The half-lives of the enantiomers were similar but the area under the curve (AUC) values of the more potent (+)-enantiomer were about three times higher than those of its optical antipode.

3.5. Ligand exchange phases

In ligand exchange phases, an amino acid such as L-proline is bonded to silica gel and the resulting phase is treated with copper sulphate solution. The separation is based on the formation of an enantioselective ternary complex between amino acid (fixed ligand), copper ion and the analyte (mobile ligand), on the stationary phase. The differences in stabilities between complexes with D- and L-form of the analyte lead to the separation of the enantiomers. Factors affecting the selectivity and efficiency of the separations include the pH, the ionic strength of the mobile phase, and the temperature. For the separation to be successful the sample molecule must have two polar functional groups with the correct spacing which can simultaneously act as ligands for the copper ion. For this reason the ligand exchange approach works very well for underivatised racemic α-amino acids with their amino and carboxyl groups and similar other compounds [11,13].

The usual mobile phase is 0.25 m*M* aqueous copper sulphate solution buffered to an appropriate pH. The presence of copper sulphate in the mobile phase prevents a loss of copper ion from the stationary phase. Strongly acidic or basic samples should be avoided or derivatised. Ligand exchange columns have been successfully used for the optical resolution of mixtures of amino acid racemates [50].

3.6. Experimental conditions

As described in the previous sections, chiral separations are very sensitive to changes in temperature, pH, ionic strength, polarity and composition of mobile phase. A small change in these parameters causes a drastic change in retention and resolution. This is because chiral recognition and resolution require highly specific, simultaneous interactions [8] and hence careful control of experimental conditions is necessary for reproducible results. In this respect a greater understanding of molecular interactions is useful in developing chiral separation methods.

Enantiomeric drug analysis by HPLC can become more complicated than the conventional HPLC drug analysis for several reasons. For example, the racemic impurities in the drug and/or internal standard could give false enantiomeric peaks. The choice of internal standard is therefore crucial in enantiomeric drug analysis, and some workers prefer not to use internal standard to avoid complications. Further problems can arise particularly in biological samples since a pair of peaks can be obtained from an enantiomeric metabolite. For identification of peaks and calibration it is necessary to have pure samples of enantiomers of drugs and metabolites. This could make the method more expensive and/or time-consuming since enantiomers of drugs or metabolites are either expensive or not available in which case they have to be synthesized, adding time and cost to analysis. Low efficiency and low flow-rates (usually 0.8 ml/min or less) associated with chiral columns are indicative of a slow mass transfer between phases which results in comparatively long retention times. Due to this reason, chiral HPLC is usually slower than conventional HPLC, however, this is compensated somewhat by the high selectivity of some of the CSPs. Since supercritical fluid chromatography (SFC) is known for its speed and high efficiency, better separations and shorter analysis times could be achieved if chiral phases were used with SFC.

3.6.1. Detectors. Currently conventional UV or fluorescence detectors are used in the majority of the HPLC investigations of chiral drugs. However, these detectors cannot confirm the indentity of the isomers as they are eluted. Optical activity based detector can give information on the elution order of the antipodes which could be very useful in understanding the recognition mechanism of the CSP. For peak confirmation, diode array detector, and for information on the chirality of the eluates, polarimetric or circular dichroism (CD) detector would be ideal. Unfortunately, these detectors are costly and have limited sensitivity particularly for biological samples, so at least for the present, conventional UV–fluorescence detectors are quite satisfactory so far as quantification of enantiomers is concerned. In the absence of specific detectors, the peaks can be confirmed separately by recording CD spectra on eluted fractions of isomers [33,51], or by injection of a solution of pure enantiomer if available [34]. Ber-

TABLE 1

CHIRAL STATIONARY PHASES FOR ENANTIOMERIC DRUG ANALYSIS

Group	Mobile phase	Applications
DNB derivatives		
DNB-phenylglycine	Normal-phase solvents	Benzodiazepinones, barbiturates, succinimides, propranolol and primaquine
DNB-leucine	Normal-phase solvents	As above
Proteins		
α_1-Acid glycoprotein	Aqueous buffer	Acidic and basic drugs, analgesics, β-blockers, nomifensine, disopyramide, chloroquine and its metabolite
Bovine serum albumin	Aqueous buffer	Amino acids and their derivatives
Cyclodextrins (CD)		
α-, β-, γ- and acetylated CD	Reversed-phase solvents	Several drugs such as propranolol, chlorthalidone, ketoprofen, hexobarbital and phenytoin metabolite
Synthetic polymers		
Cellulose esters, carbamates	Normal-phase solvents, polar solvents (water–alcohol)	Benzodiazepines, β-blockers, alkaloids
Polymethacrylates	Normal-phase solvents, polar solvents (water–alcohol)	Nilvadipine
Ligand exchange	Buffered copper sulphate solution	Amino acids

tucci et al. [25] have used CD and UV detectors (connected in series) in conjunction with a quinine-based CSP to determine the stereochemical purity of drugs such as benzodiazepines.

Recently a sensitive polarimetric detector for HPLC using a laser diode light source has been developed [52] but is not yet commercially available. The laser is focussed on to a micro-HPLC cell with a volume of 1–20 μl, thus enabling to characterise the optical activity of just a few microliters of sample. Further tests are being carried out to demonstrate the utility of the detector in routine quality control of optically active compounds.

4. CONCLUSIONS

HPLC with chiral bonded phases offers new approaches to studying the pharmacological and metabolic properties of drug enantiomers via their determination in biological fluids and to determining optical purity of drugs. Isomers that were previously thought to be difficult to separate by conventional HPLC can now easily be resolved with the aid of CSPs. CSPs that have been used to advantage in the HPLC resolution of drug enantiomers are summarised in Table 1.

Routine enantiomeric drug analysis is still very much in its infancy but its use can be be expected to increase in the future as new and improved stationary phases are developed for this purpose offering more selectivity in enantiomeric separations. Those CSPs which are already in the market place [13] are relatively new and their performance in general and potential in drug analysis in particular are yet to be fully assessed. Although not all racemic mixtures can be separated on any given chiral column, it would be nice to see the emergence of an ideal or near-ideal chiral packing (along the lines of C_{18} in RP chromatography) useful in many applications in drug analysis. At present it is not easy to select an appropriate chiral column for a particular separation problem. From the analytical point of view, there is a shortage of chiral compounds (standards) which are required for calibration and confirmation purposes during analysis as is a necessity for more specific and sensitive detectors that provide information on the chirality of the eluted solutes. Since the different behaviour of enantiomeric drugs is now well-recognised, the regulatory bodies may in future demand more data on the individual isomers in racemic drugs or even specify that certain drugs should be sold as optically pure compounds.

At present chiral compounds and bonded phases are by no means cheap since the development costs are formidable. Further studies involving the theory and mechanism of chiral recognition could provide useful routes to the synthesis of a new generation of CSPs. This coupled with an increase in demand for chiral chromatography and for optically pure compounds would certainly increase the availability of chiral columns and compounds at economical price.

5. SUMMARY

The development of chiral stationary phases for HPLC has resulted in renewed interest in methods for the separation of drug enantiomers. This paper provides a brief overview of some of the more recent approaches to the direct resolution of drug enantiomers by HPLC with particular emphasis on their quantification in biological fluids.

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