Updates in the Technology and Application of Chiral Stationary Phases

Utilization of chiral separation methods remains unabated in new drug development. Activity in the creation of new chiral stationary phases has decreased and has been replaced largely by new method developement strategies such as screening multiple chiral columns with automated systems, biocatylic conversion methods, and new highly efficient catalysis for the efficient production of single enantiomers. Established technologies have been dramatically improved and there have been new attempts at replacing silica as a base material. In addition, some new concepts for chiral recognition have been introduced. In general, activity in the separation of chiral molecules remains high.

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Approximately 60% of known drug substances in development or production have one or more chiral centers. Often only one of the enantiomers is physiologically active, while in some instances, the other enantiomer has some detrimental side effects or other physiologically undesirable characteristics. Knowing this, the U.S. Food and Drug Administration (FDA) requires new chiral drugs to be analyzed for chirality. Such requirements have spurred a wave of activity in two areas: first, in the development of analytical procedures, namely high performance liquid chromatography (HPLC) and to a lesser extent capillary electrophoresis (CE), to quantify drug enantiomers; and second, in the synthesis of single-enantiomer drugs.

On the analytical side, a recent informal survey of several top pharmaceutical companies conducted by the authors indicated that the demand for chiral separations remains unabated. Many pharmaceutical companies now are screening routinely for chiral selectivity with column switching systems that screen four, six, or eight chiral stationary phases on a 24-h basis. On the synthesis side, in an effort to decrease the downstream processing pressure generated from this high level of activity, specialty chemical companies have geared their expertise to the efficient production of single enantiomers as building blocks for new pharmaceuticals in the drug-discovery process. It is thought that this approach will alleviate the need for preparative purifications, currently a stumbling block in drug development.

Chiral Stationary Phases and Mobile Phase Types

Chiral stationary phases are designed to provide maximum selectivity in certain mobile-phase environments. It matters little if the bonds are covalent and the chiral stationary phase is stable in all mobile-phase types. It is important that the required functionality is present in the chiral stationary phase to allow for selectivity to occur. Although the nomenclature is not standardized for chiral stationary phases, there has been a tendency, as in other forms of HPLC, to refer to experiments performed in organic solvent as normal phase and to refer to experiments performed in aqueous solvents as reversed phase. Phases of the π electron type require a nonpolar solvent such as hexane to enable π electron sharing or anchoring of the analyte to the chiral stationary phase. In this normal phase mode, hydrogen bonding simultaneously occurs between a polar group on the analyte and a suitable acceptor site on the stationary phase (a polar alcohol is used with the hexane to modulate the

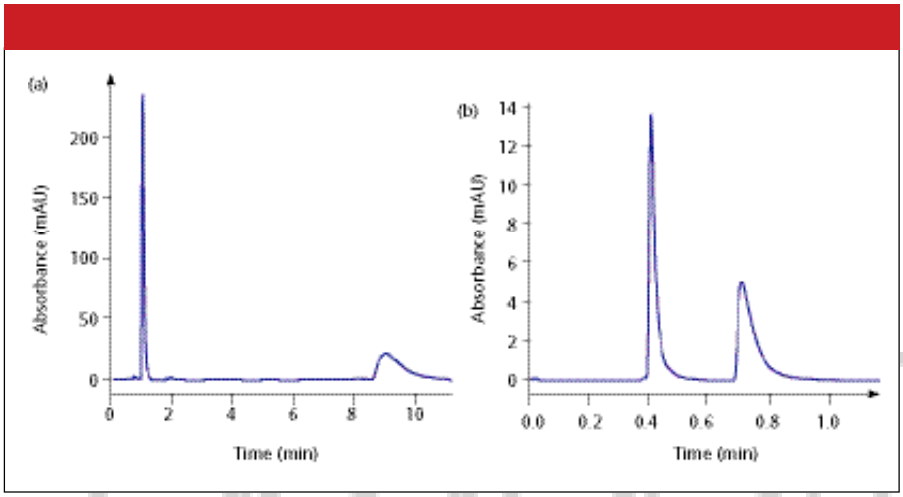

Figure 1: Chiral compounds on (a) porous and (b) nonporous zirconia-based DNB-L-Leu anchored with APPA. Conditions: mobile phase: 99:1 hexane–isopropanol; probe solute (±)1-naphthyl leucine ester. (Courtesy of X.Xin, ZirChrom Separations.)

hydrogen bonding interaction). In the reversed-phase mode, anchoring involves either inclusion complexation, very strong hydrogen bonding, or ion exchange; water is the primary solvent, an organic solvent modulates the inclusion and hydrogen bonding, and a buffer controls the ion-exchange interaction. A third mode relates to cyclodextrin phases and is referred to as the polar organic mode. The current use of this terminology by others in the field is described now as a combination of polar alcohols, typically methanol–ethanol, which results in a modified inclusion and simultaneous control of hydrogen-bonding effects. When ion-exchange sites are present in the chiral stationary phase as with CHIROBIOTIC phases (Advanced Separation Technologies [Astec], Whippany, New Jersey), then the presence of a volatile salt or low concentrations of both acid and base are required for the ion-exchange function. At the same time, the polar alcohol controls the hydrogen bonding. This latter mobile phase now is referred to as the polar ionic mode to differentiate it from the polar organic mode. Many recent chiral stationary phase developments focus on the ability to operate in many or all of these modes, primarily because of the broad range of molecule types and polarities confronted by the analyst.

Phases in Current Use

The number and type of new technologies entering the field of chiral separations appear to have slowed, while at the same time, an expansion of older technologies has continued to increase. Cellulose and amylose chiral stationary phases, trademarked as CHIRALCEL and CHIRALPAK (Daicel Chemical Industries, Osaka, Japan), respectively, Chiral Technologies, Inc. (Exton, Pennsylvania) continue to dominate chiral separations. The use of these phases is especially strong in drug discovery, and their growth has been generated by the addition of new chiral functionalities as well as application innovations. Two older technologies also have proliferated in the field, including the π-electron phases commonly referred to as the "Pirkle type" (named after William Pirkle, University of Illinois, Urbana, Illinois) and cyclodextrin chiral stationary phases (not referred to as the "Armstrong type," even though Daniel Armstrong of Iowa State University [Ames, Iowa] was an early pioneer in their development [1]). It has been noted by the authors that 14 companies now produce more than 36 varieties of cyclodextrin liquid chromatography (LC) phases. The number of Pirkle types available defies counting, primarily because of the subtle phase differences that exist and which lead to overlaps that are really not significant from an operational standpoint. In researching the literature, it is apparent that there is a strong desire to replace silica as the base support or to create entirely new effective chromatographic technologies for the chiral recognition mechanism; technologies that are more broadly applicable, cheaper to produce, and easier to use. Recently, ZirChrom Separations (Anoka, Minnesota) and Shiseido Fine Chemicals (Tokyo, Japan) have addressed the first of these points, while the CHIROBIOTIC macrocyclic glycopeptide phases (Advanced Separation Technologies) addressed the latter more profoundly.

New Base Materials

The newest company to enter the field of chiral separations, ZirChrom Separations, has made a strong attempt to turn the tide from silica- and polymer-based chromatographic supports to its pH-stable zirconium base. In addition to its growing list of standard phase technology applied to the zirconium base, ZirChrom recently has announced a series of "Pirkle, brush-type" π electron–based ligands (2). Although the need for pH stability with a mechanism primarily functioning in a stable normal-phase environment has yet to be determined, peak efficiency and increased selectivity seem to have the most important benefits. The company uses the typical aminopropyl linkage with a phosphonic acid group forming the Lewis base anchor and then attaches, through the amino function, a number of chiral selectors such as 3,5-dinitrobenzoyl leucine. High concentrations of trifluoroacetic acid, normally used as an ion suppressor, had no effect on the stability of this chiral stationary phase, nor did it cause any deterioration in performance, as was demonstrated when compared to a model silica-based material. A second Pirkle-type ligand, 3,5-dinitrobenzoylphenylglycine, also has been introduced. 3,5-Dinitroben-

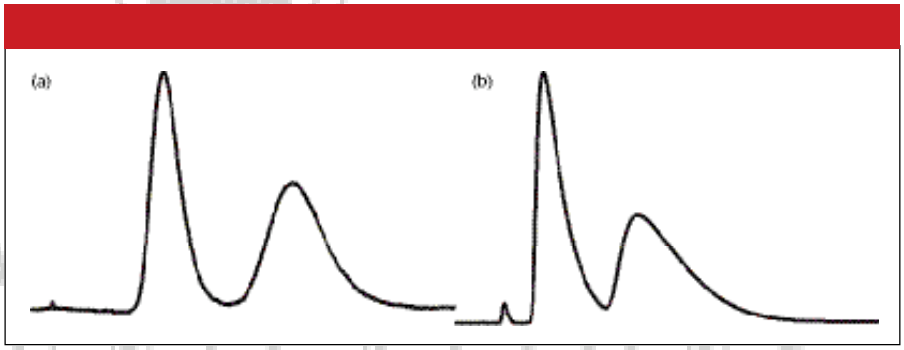

Figure 2: Enantiomeric separation of chlormezanone. Column: 250 mm × 4.6 mm Ceramospher RU-2; sample load: (a) 0.1 mg (R_s: 1.65) and (b) 50 mg (R_s: 1.12). (Courtesy of Dr. F. Kanda, Shiseido Fine Chemicals)

zoylphenylglycine and 3,5-dinitrobenzoylleucine have been bonded to both porous and nonporous zirconium particles. It was found that substantial reduction in analysis time could be obtained using the nonporous structure. An 11-fold reduction in separation time can be seen for this comparison in Figure 1.

From Shiseido Fine Chemicals (Tokyo, Japan), a new and very interesting concept in chiral recognition has been announced. After the preparation of a novel spherical sodium magnesium silicate particle, the resulting media are used to perform an ion-exchange linkage between the sodium in the matrix and tris (1,10-phenanthroline) ruthenium(II) complex. This product, trade-named Ceramospher, can function in both normal-phase and aqueous chiral separation modes. However, two different versions of the column must be used. The column designated RU-1 operates in the normal-phase mode, using predominately methanol as the mobile phase, sometimes with the addition of either acetic acid or triethylamine as modifiers. In an aqueous mode, the RU-2 form again uses methanol as the primary solvent that is further modified with water, acid, or base as the mobile phase, but published separation data have indicated that it also can be used in the normal-phase mode (3). The chiral stationary phase appears to function based upon π–π interactions and steric effects from the optically active ruthenium complex. As a result of the large specific surface area, this chiral stationary phase has a good capacity for increased amounts of racemate. From a recently published list of separation data for 52 compounds (2), the selectivity was in the range of 1.03–3.19, with an average of 1.43. The media, however, demonstrated very high sample capacity with as much as 50 mg on an analytical scale column (250 mm × 4.6 mm), as can be seen in Figure 2. The stationary phase was unaffected by exposure to a wide variety of solvents, but 100% methanol seemed to be the most generally useful mobile phase. The column also was shown to be stable over a wide temperature range. In the analytical data reported, temperature appears to play a key role and was widely used, with temperatures as high as 50 °C in both the normal- and reversed-phase modes. Because no retention data were given, it was not possible to access the efficiency of the separation.

The quinine and quinidine carbamates originally developed by Wolfgang Linder, University of Vienna (Vienna, Austria), and sold through Bischoff Chromatography (Leonberg, Germany) as ProntoSil Chiral AX columns. This company recently announced the availability of these phases in a micro-LC format for the separation of peptide stereoisomers employing the ion-exchange mechanism commonly associated with these phases. The QN-1 CSP designates the quinine carbamate phase, while the QD-1 designates the quinidine carbamate. This new product and application recently was described in a publication (4). The scale-down has not sacrificed the resolution power of these phases, but an N-terminal protection group was necessary to eliminate the zwitterionic nature of these types of analytes. Several N-terminus groups were investigated. The N-tag, in addition to increasing detectability, does take part in the separation mechanism. Two types of mobile phases were investigated, a hydro-organic mobile phase, usually methanol–0.5 M aqueous ammonium acetate, and a polar organic mobile phase consisting of acetonitrile–methanol with 400 mM acetic acid and 4 mM triethylamine. The highest stereoselectivity occurred with the dinitrobenzoyl and dinitrophenyl derivatives in the reversed-phase mode. The published information indicates that peptides with more than four amino acid residues would be difficult or impossible to resolve. However, the enantiomers from one to four amino acid residues all could be resolved to baseline.

Developments in Cellulose and Amylose Chiral Stationary Phases

Chiral Technologies, Inc. (Daicel, Exton, Pennsylvania) has announced the completion of the H series of both the cellulose and amylose derivatives. The H series is based on a 5-µm particle and includes chiral analytical and semipreparative columns for LC and supercritical fluid chromatography (SFC). It has been noted in the published reports on these phases that the usual additives for SFC operation, such as 0.1% trifluoroacetic acid, is not required in the mobile phase for acids, eliminating the possibility for esterification during work-up (5). This phenomenon has been attributed to the somewhat acidic nature of the carbon dioxide used as the supercritical mobile phase. Product recovery from preparative and semipreparative SFC columns after conversion of the carbon dioxide to a gaseous state leaves the analyte in pure alcohol modifier. A process for the production of flurbiprofen shows the generation of single enantiomer at the rate of 2 kg of enantiomer/kg of chiral stationary phase (CHIRALPAK AD-H) per day at 99% enantiomeric excess.

At Pittcon 2004 in Chicago, Illinois, Chiral Technologies launched its first in a series of immobilized polysaccharide chiral stationary phases designated Chiralpak IA. The objective of this new technology is to increase solvent flexibility and extend durability. Applications were shown using a variety of solvents including ethyl acetate and methylene chloride. No comparison of selectivity to the original amylose-coated chiral stationary phase was available and no comments were given on the effect of bonding on overall selectivity.

Cyclodextrin Developments

The latest entrant into the field of cyclodextrin technology is YMC (Kyoto, Japan). YMC has focused primarily on preparative applications. Two versions have been offered, the native beta and a permethylated version in particle sizes of 10, 20, and 50 µm. A load capacity of 100 mg for chlorthalidone on a semipreparative column (250 mm × 20 mm) was noted. Most of the applications on these two phases have been in the reversed-phase mode. An occasional application in a normal-phase mode was noted but only on the permethylated version. Shiseido also has introduced a cyclodextrin phase in the form of a phenylcarbamated derivative of β-cyclodextrin to complement its ruthenium-complex column cited earlier.

Macrocylic Glycopeptide Developments

One of the advances in the CHIROBIOTIC macrocyclic glycopeptide phases (Advanced Separation Technologies [Astec]) has been the introduction of the aglycone forms of these broadly used chiral ligands. The CHIROBIOTIC TAG, which is the aglycone form of the bonded teicoplanin, has been shown to have unique selectivity not only for amino acids and N-blocked amino acids but also for many of the amino acid building blocks used in drug discovery in the preparation of chiral pharmaceuticals. One of the early indications of the phase usefulness was that it could resolve neutral racemates in single solvents such as methanol, ethanol, and acetonitrile. This information has now translated into an excellent SFC phase. A study of a set of 111 compounds recently has been published that included heterocycles, analgesics, β-blockers, and sulfoxides (7). The CHIROBIOTIC TAG and CHIROBIOTIC T phases accounted for 92% of the

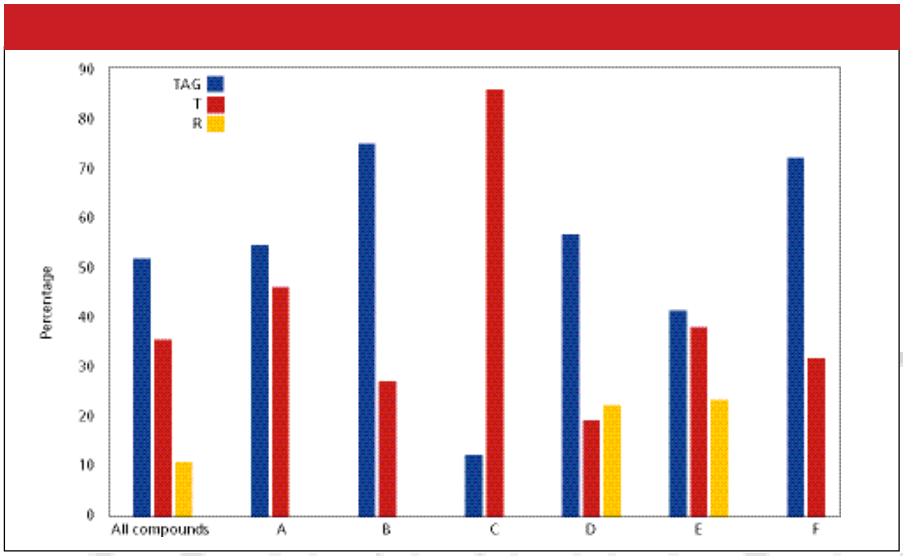

Figure 4: Percentage of successful SFC separations for various classes of compounds on CHIROBIOTIC phases. Columns: CHIROBIOTIC TAG, CHIROBIOTIC T, CHIROBIOTIC R; A = heterocyclic compounds, B = chiral acids, C = β-blockers, D = chiral sulfoxides, E = N-blocked amino acids, F = native amino acids, T = teicoplanin CSP, TAG – teicoplanin aglycone CSP, and R = ristocetin A CSP. (Courtesy of D.W. Armstrong, Iowa State University)

separations. All separations were done in less than 15 min and 70% were done in less than 4 min (Figure 4). The vancomycin aglycone did not demonstrate as broad an increase in chiral selectivity but proved to make a good contribution to the overall chiral tool box.

The CHIROBIOTIC technology has been useful for a variety of compound types as well as the separation of the chiral and achiral metabolites in a variety of mass spectrometry (MS)–compatible mobile phases. For LC–MS platforms, the number of published applications on these stationary phases in the drug-metabolism area currently exceed all other stationary phases combined.

Several new chiral stationary phases were presented by Astec at Pittcon 2004. Two product-line extensions designated CHIROBIOTIC V2 AND CHIROBIOTIC T2 were introduced that yielded higher selectivity and capacity but only in the polar organic and polar ionic modes. Capacities in excess of 150 times the standard product were presented. Selectivity in other modes for this product were diminished or completely eliminated. These products were specified for preparative and LC–MS applications primarily. A new bonded polymeric chiral stationary phase was introduced exclusively for normal-phase use. It had is best selectivity and efficiency in methylene chloride–methanol mobile phases. It was claimed that the mechanism was only hydrogen bonding and some steric effects created from the polymer matrix.

Conclusions

All of the previously cited technologies address some issue within the chiral separations area, but the diversity of new chemical structures continues to push the frontiers of current separation technologies. It must be stated that awareness of new technologies does not necessarily translate into progress in the current climate of the pharmaceutical industry. This dilemma exists because the time it takes to understand these technologies is overshadowed by the pressing need to get the job done. It turns out that in today's market, an 80% rate of success is more than acceptable if it is done in one day as opposed to a 100% success rate in 10 days.

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