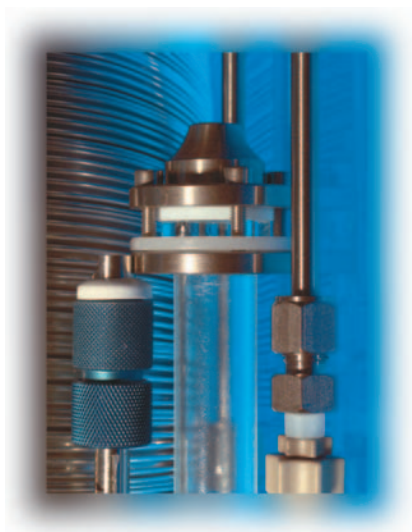


Updates in the Technology and Application of Chiral Stationary Phases

Chiral separations remain high on the priority list of drug developers. Liquid chromatography and supercritical fluid chromatography continue to vie for dominance in the chiral separation market but might be reaching the state of complementary methodologies. New separation technologies and new advances in current products continue to elevate the state of the art of chiral separations. Automation in both screening for chiral selectivity and preparative purifications has helped make significant gains in productivity in this area.



We last updated the current status of chiral stationary phases in 2004 (1). As the number of new drug candidates that are chiral increases from year to year, so has their complexity. In addition, the number of compounds with multiple stereogenic centers has increased, further complicating the separation process. The increased need to develop rapid and effective separation methods has been the result of two factors. First, government regulations have required the purification and biological testing of the individual enantiomers of potential chiral drugs. Second, there has been an increased emphasis on introducing chirality in the drug discovery platform to enhance drug-receptor binding and selectivity. Given the number of available chiral stationary phases (>200), and the lack of a definitive direction in choosing the ideal phase, screening protocols now have become de rigueur. Automation in analysis and purification systems — for example, mass-directed prep — also has been explored as a centralized specialist group or as a general analytical talent within the drug discovery or synthesis groups to increase the needed efficiency and throughput (2).

Chiral Selectivity: Separation Methods

A number of different separation methodologies have been explored to date in an attempt to bring the necessary efficiency to this difficult separation problem. Capillary electrophoresis (CE) and capillary electrochromatography, while generating a

large volume of published papers, have not succeeded in bringing a stable efficient solution to the problem. However, one company has reported the successful conversion of CE data to preparative purifications (3). The key to success was the use of derivatized cyclodextrins from the same manufacturer that produced the bonded media. Capillary gas chromatography (GC) has served well the analysis of chiral building blocks and evaluating chiral raw material sources (4) but the limitations of analyte volatility and stability have limited the use of this technique. In addition, it does not have preparative capabilities. High performance liquid chromatography (HPLC) has proven to be the best methodology to deal with the diversity and complexity of the problem. In liquid phase methodology, two basic modes of operation have developed; HPLC and super- and subcritical fluid chromatography (SFC).

LC Methods

The majority of pharmaceutical companies are now screening routinely for chiral selectivity with column switching systems that screen four, six, or eight chiral stationary phases on a 24-h basis in a specialized service facility. In some other companies, decentralization has occurred so that the separation activity is closer to the source. In these situations, simple “walk up” HPLC systems utilizing a limited number of columns have been set up. Under these conditions only the most difficult separation problems are sent off to the specialist. To enhance the capabilities of both the routine

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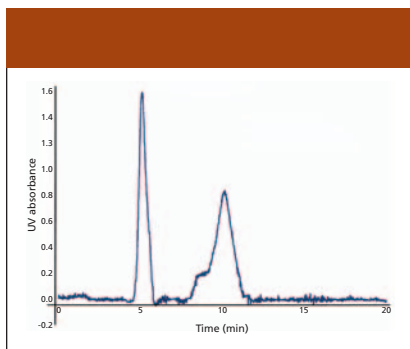


Figure 1: Separation of 2-methyl-pentanol enantiomers on an ESP Normal Phase chiral column. mobile phase: 99:1 hexane: iso-propanol; flow rate: 0.5 mL/min. Injection: 50 wt% in ethanol. (Courtesy of Evolved Nanomaterial Sciences.)

analyst and the centralized specialist, two fully automated, parallel column screening systems were introduced last year. In these situations, the overall efficiency and productivity for solving chiral separation problems appears to have increased fourfold. Fast screening for selectivity is obviously the key. The greater the breadth of choices that can be screened, the better the outcome will be to satisfy the variety of application needs in addition to what is required for preparative purifications.

SFC Methods

The on again, off again potential of SFC has been debated with renewed intensity. There is no doubt that this technique offers speed and very much reduced solvent costs for both purchase and disposal of mobile phase components but, SFC has demonstrated its limitations to normal phase type separations. A number of laboratories once solely dedicated to SFC now have changed to a 50–50 split with conventional LC. It is best to say this technique is complementary to LC (4). That said, the cellulose and amylose phases, especially Daicel's, Tokyo, Japan, CHIRACEL OD, OJ and CHIRALPAK AD and AS, have played a major role in the chiral separation of neutral and less polar molecules. For the resolution of more polar racemates, Advanced Separation Technologies' (Astec, Whippany, New Jersey) CHIROBIOTIC T, TAG and R and the polymeric phases P-CAP and P-CAP-DP have been complementary.

New Concepts in Chiral Stationary Phases

A new concept in chiral separation recently has been announced by Evolved Nanoma-

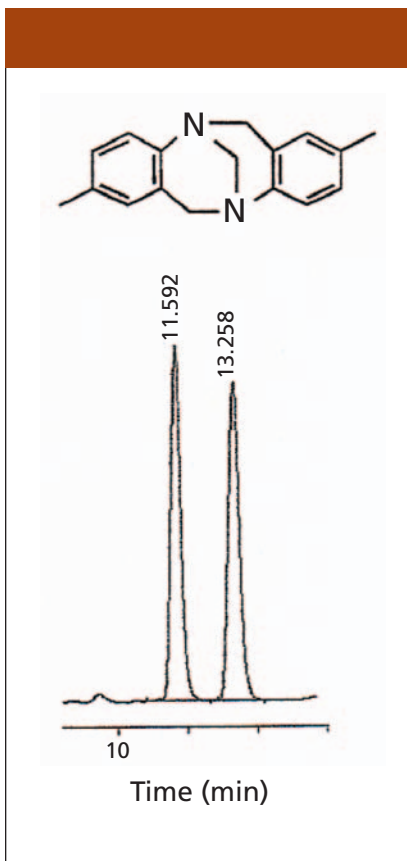


Figure 2: Separation of Troger's base on a Sepapak-2 Derivatized Cellulose chiral column. Mobile phase: 9:1 n-hexane–2-propanol; flow rate: 0.5 mL/min. (Courtesy of Sepaserve GmbH.)

terial Sciences, Inc. (ENS) (Cambridge, Massachusetts). It is based upon the spacial separation of chiral molecules rather than the chemical mechanism typically referred to as “three points of interaction.” This novel universal stationary phase is, therefore, a true shape based selector that employs nanostructured channels within the particle interior. The stationary phase resolves chiral molecules based upon morphology at the molecular scale.

The first stationary phase developed by ENS for chiral separations, designated ESP, addresses the need for simplicity in chiral methods development and broadens the pool of chiral stationary phases for LC to now include separations of volatile compounds, low molecular weight compounds, hydrocarbons and other chemical classes that are poorly addressed by conventional chiral LC column technologies. For example, one normal phase column with ESP can separate chiral alcohols, amines, terpenes, propargylic alcohols, carboxylic acids, aliphatic alcohols, as well as some of the more complex pharmaceutical mole-

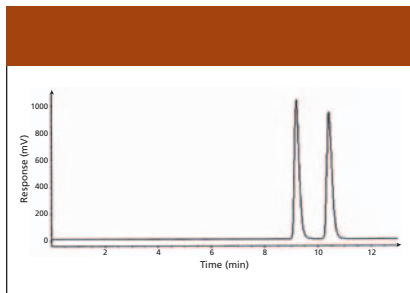


Figure 3: Separation of atropine enantiomers on a Chiralpak 1B column. Mobile phase: 65:35:0.1 n-hexane–chloroform–ethanolamine; flow rate: 1.0 mL/min. (Courtesy of Chiral Technologies.)

cules such as propranolol and thalidomide. It is difficult, at this point in time, to evaluate the shape restrictions that might exist within a given design or to evaluate the efficiency with which the separations are achieved. This can be an ideal preparative media rather than an analytical tool but it is definitely a product to watch.

Advances in Stationary Phases Currently in Use

Cellulose and amylose chiral stationary phases: Sepapak-2 is a new member of the most widely used polysaccharide family of chiral stationary phases for HPLC enantioseparations and is available from Sepaserve GmbH, (Munster, Germany). The derivatized cellulose, however, is a 3-chloro-4-methyl carbamate instead of the typical 3,5-dimethyl carbamate. As a result of this modification and new silica this classic stationary phase exhibits new advantages as follows:

- It is able to resolve all 10 of the established test racemic compounds for these types of materials.
- The single column can operate in both normal- and reversed-phase modes.
- It is claimed to be stable at operating pressures as high as 300 bar (4400 psi).

Chiral Technologies, Inc. (Daicel, West Chester, Pennsylvania) has announced the introduction of the second in the series of bonded polysaccharide phases designated Chiralpak 1B. The introduction last year of the Chiralpak 1A as the bonded 3,5-dimethylphenyl carbamate derivative of amylose, corresponding to the coated version, Chiralpak AD. This column now has been followed by the second most popular chiral stationary phase which is the 3,5-dimethylphenyl carbamate derivative of cellulose trademarked as Chiralcel OD. These two bonded phases for some time have been

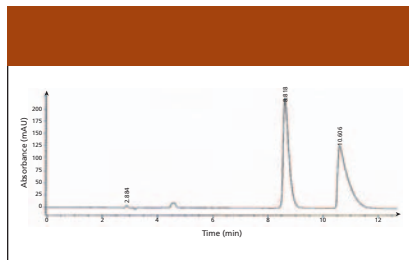


Figure 4: Separation of *trans*-stilbene oxide enantiomers on a Cyclobond I 2000 DNP chiral column. Mobile phase: 25:75 acetonitrile–0.1%TEAA (pH 4.1); flow rate: 1.0 mL/min.

made available only in the 5- μ m analytical format but it was recently announced that the 20- μ m preparative version will be available in the first quarter 2006. The stated benefits of these two bonded versions have been the extended solvent flexibility and extended durability over the coated versions. Applications on these phases demonstrate the use of solvents like dichloromethane, methyl-*tert*-butyl ether and chloroform. At the ISCD 2005 meeting in Pharma, Italy, it was reported that there is a lack of continuity of the method development process from the coated versions. It appears that these new bonded versions could be relied on for the resolution of existing problems with coated versions rather than as a replacement (6).

Quinine and quinidine phases: Chiral Technologies also announced the availability of two new chiral stationary phases, the Chiralpak QD-AX and QN-AX based upon a licensed agreement with Professor Wolfgang Linder of the University of Vienna. These phases, originally marketed under another tradename, are based upon the bonding of derivatized quinine and quinidine. They are being promoted as ideal selectors for chiral acids in reversed-phase and polar organic mode solvents and are stated to be applicable to both LC and SFC. It was further noted in an announcement that all 5-mm stationary phases will be available in a 150 mm \times 0.3 mm microflow capillary format to meet the demand for smaller sample amounts and lower mobile phase consumption.

Macrocyclic glycopeptide CSPs

Astec announced the introduction this year of a four column chiral screening kit containing the CHIROBIOTIC stationary phases V, T, TAG, and R with a method development protocol that fosters screening in four mobile phase types on

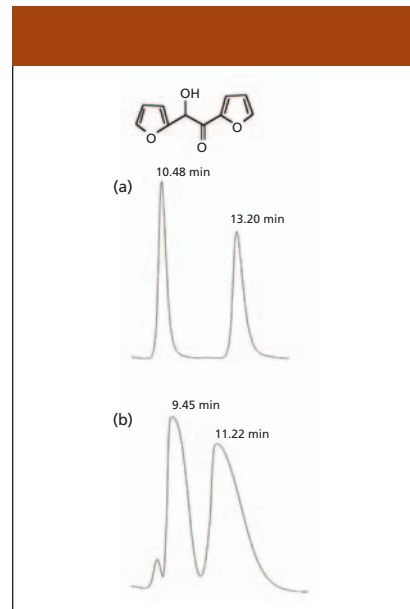


Figure 5: Separation of furoin enantiomers at different loading levels of (a) 8 μ g and (b) 1000 μ g on a P-CAP chiral column. Mobile phase: 20:80 ethanol–hexane; flow rate: 1.0 mL/min; injection volume: (a) 0.2 μ L (4 μ g/ μ L), (b) 50 μ L (20 μ g/ μ L).

each column to produce data that could be applied to a variety of applications like liquid chromatography–mass spectrometry (LC–MS), dissolution analysis and preparative applications. The columns in the screening kit are available in 100 mm \times 4.6 mm dimensions.

Astec also announced the availability of an amino acid and peptide Handbook that utilizes the macrocyclic glycopeptides, especially the CHIROBIOTIC T 2 for the separation of peptides that differ by a single amino acid or the chirality of a single amino acid anywhere in the peptide chain. With these phases natural or synthetic amino acid or N-blocked amino acid can be separated as well.

New cyclodextrin technology: Astec has announced the availability of two new cyclodextrin derivatives. The first is an extension of the popular CYCLOBOND I 2000 RSP product and is designated CYCLOBOND I 2000 HP-RSP. This is specified as a change in substitution level on the β -cyclodextrin secondary hydroxyl groups and the type of linkage used in the bonding chemistry that has a dramatic effect in extending the selectivity of this phase. It has enhanced the resolution of the conazole type anti-fungal agents and a number of other important separations like methadone and its metabolite EDDP, Jacobsen's catalyst and a wide variety of chiral amines.

A totally new derivative also was announced, trademarked CYCLOBOND I 2000 DNP, a long sought after derivative that introduces a π -acidic dinitrophenyl ring enhanced with a trifluoromethyl functional group onto β -cyclodextrin. In the reversed phase mode, this product dramatically has extended the selectivity of cyclodextrin technology and is seen as a replacement for the native product itself (CYCLOBOND I 2000) as well as other cyclodextrin based derivatives. The demonstrated separations have been fast, efficient and the stationary phase appears to be very stable.

New Zirconium-Based Materials

ZirChrom Separations, Inc. (Anoka, Minnesota) recently has expanded its line of zirconium-based chiral stationary phases to five Lewis acid–base anchored chiral selectors. The list now includes the chiral selectors, (*S*)-3,5-dinitrobenzoyl-leucine, (*R*)- and (*S*)-N-[1-(1-naphthylethyl)succinamic acid] and (*R*)- and (*S*)-3,5-dinitrobenzoyl-phenylglycine. These are available for analytical as well as preparative applications. The strong Lewis acid sites on the surface of the zirconia allow reversible attachment of the chiral stationary phase in a two-step approach: an appropriate tethering group is attached to the zirconia surface, and the chiral selector is covalently attached to the tethering group using amide bond formation. This approach allows a contaminated stationary phase to be removed by rinsing the column with base and then the column can be easily regenerated by repeating the simple two-step process explained previously. It is claimed that these selectors have similar chiral selectivity to silica-based CSPs with obviously longer stability and within an expanded pH range.

New Polymeric Stationary Phases

Developed at the University of Rome by Dr. F. Gasparini and licensed to Astec, this new polymer methodology is designed for use in normal-phase chromatography. Introduced as the P-CAP phases, an acronym for polycyclic amine polymer, this polymerization reaction has been controlled to produce a chiral phase from 1,2-cyclohexyldiamine through an acrylo-polymerization reaction. The phase relies on hydrogen bonding only and whatever steric effects are obtained from the resulting

matrix. This stationary phase appears to have broad solvent selectivity and can, therefore, utilize a wide variety of organic solvents. A second version also recently has recently been introduced labeled P-CAP-DP for polymeric cyclicamine polymer-diphenyl. In this phase, aromatic groups are inserted off the amine functions further enhancing the chiral selectivity with π – π interactions and any attendant steric effects that result from the insertion of these bulky groups. The advantage of these phases is their applicability for preparative purifications where a variety of chemical reaction solvents can be used directly in obtaining the chiral separation. As a repeating chiral ligand these phases have good capacity and are useful in both LC and SFC.

Conclusions

Routine chiral separations are now possible given the advances in both product performance and user knowledge. There are still chiral separations that beg solutions but new and innovative technologies are still being developed. Through new screening protocols great gains have been made in the speed and number of chiral separations that are accomplished each day. Automation, as in most areas of drug discovery, has found its way into chiral separations as witnessed by recent instrument introductions.

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