

Method Development for Chromatographic Chiral Compound Analysis and Isolation Using the New Immobilized Stationary Phase Technology

By Geoffrey Cox and David Ellis

Chiral Technologies, Inc.,
West Chester, Pennsylvania

INTRODUCTION

Enantiomers of chiral compounds can have dramatically different pharmacological effects thereby making the ability to assess and isolate pure enantiomers of vital importance to pharmaceutical developers. Chiral chromatography is traditionally used as the stereoselective separation technique, with polysaccharide-based chiral stationary phases (CSP) as the media of choice for over 20 years. Although these conventional media have unparalleled application range and versatility, their main limitation has been the inability to tolerate a certain range of solvents. Chiral chromatographers therefore have had to exercise extreme precaution to avoid even small quantities of incompatible solvents that can rapidly degrade or destroy a column.

A new product line based on proprietary immobilization technologies (Chiral Technologies' Platinum Series CHIRALPAK® columns) was developed that safely accommodates virtually any organic solvent as a mobile phase or mobile phase component. The CSPs derived using these technologies exhibit remarkable stability, separation reproducibility, and durability when used in normal phase, reverse phase, and SFC modes. The ability to use an expanded range of solvents for mobile phase and sample dissolution, as well as elevated temperatures offers new possibilities for investigating novel conditions for obtaining unique separations. These new capabilities demand a new approach to method development.

METHOD DEVELOPMENT

Immobilized Solid Phase Selection

Seven years in development, the immobilization technology is available in the Platinum Series of columns—CHIRALPAK IA™, IB™, and IC™.

- CHIRALPAK IA is an immobilized CSP with a tris-3,5-dimethylphenylcarbamate derivative of amylose, which is nominally equivalent to CHIRALPAK AD-H™.
- CHIRALPAK IB is an immobilized CSP with a tris-3,5-dimethylphenylcarbamate derivative of cellulose, which is nominally equivalent to CHIRALPAK OD-H®. In contrast,
- CHIRALPAK IC is an immobilized CSP with a tris-3,5-dichlorophenylcarbamate derivative of cellulose, which is a completely unique chiral selector that does not mirror any previous product. The immobilization technologies allow the practical use of this versatile but highly soluble selector for the first time.



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A statistical evaluation of a large number of compounds indicates that, when used for chiral screening, the three columns will separate 95% of racemates. Using only a few mobile phases in the screening process delivers new and robust separations with exceptionally high success rates.

Mobile Phase Solvent Selection

The immobilized columns have been thoroughly tested for stability to most common organic solvents, particularly those in which the chiral selector is soluble. These tests indicate complete stability to these solvents; thus, although a limited range of solvents may be recommended for initial screening (see below), this is more for convenience since ultimately there are no restrictions on the solvents which may be used as mobile phase components.

Generally, CHIRALPAK IA and IB may be used in place of CHIRALPAK AD-H and CHIRALCEL OD-H in existing chromatographic methods although some small selectivity differences may be observed and the mobile phase conditions may need some small modification. Both columns offer unique selectivity when non-conventional mobile phases are used.

New chromatographic methodology is required for new chiral compounds, existing methods with unsatisfactory resolution, and for the IC column, which contains a new selector with no comparable conventional column. Table 1 provides a list of the primary solvents that may be used in a screening process to provide successful separations. Conventionally this is begun by using one of the screening mobile phases in Table 1. Following analysis of the results, a weaker or stronger solvent composition is employed to adjust retention times for reasonable analysis times. For example, if the peaks elute too quickly than one needs to use a weaker mobile phase. Note that DCM and MTBE will destroy conventional, coated polysaccharide-based chiral columns and should only be used with the new immobilized columns. Solvents in Table 2 can be used in those cases where resolution is not obtained using the primary screening solvents in Table 1. Note again that the extended range solvents will destroy conventional coated chiral columns and should only be used with the new immobilized columns.

In reversed phase mode, the columns should not be operated below pH=2 or above pH=7. The upper range of the IA and IC columns only can be extended to pH=9, provided that borate buffer is employed, and that the guard column is changed at least once every 200 injections at this pH.

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Table 1: First set of solvents for new mobile phase development

Start with running the sample using the screening solvent concentrations and adjust to weaker or stronger concentrations accordingly.

Family	Hexane/ Isopropyl alcohol	Hexane/ Ethanol	Dichloro- Methane	MTBE (methyl-tert- butyl ether)
Components	Hexane:IPA	Hex:EtOH	Hex:DCM:MeOH	Hex:MTBE:MeOH
Weaker	92:8	92:8	80:20:0	49.5:49.5:1
Screening	80:20	85:15	68:30:2	0:98:2
Stronger	70:30	75:25	0:99:1	0:85:15

Note: DCM and MTBE will destroy coated polysaccharide-based chiral columns.

Table 2: Second set of solvents for new method development

Investigate chromatographic conditions using Table 2 solvents if chiral resolution is unsatisfactory using those in Table 1.

	Extended Range		Polar Mode	
Hexane/Ethyl Acetate 70:30	Hexane:Chloro- form:Ethanol 65:30:5	Hexane:Tetra- Hydrofuran 70:30	Methanol or Methanol: Ethanol 50:50	Acetonitrile 100

Note: Extended Range solvents—ethyl acetate, chloroform, and tetrahydrofuran will destroy coated polysaccharide-based chiral columns.

Sample Dissolution Solvent

Sample solubility is a key consideration in chiral HPLC separations when scaling up from analytical, through semi-preparative to preparative columns. A wider variety of sample solvents can now be used with a greater likelihood that sufficient sample solubility can be realized, thereby enabling higher sample loads. Chlorinated solvents which are often preferred because they dissolve most organic compounds are completely safe to use with the immobilized CSPs.

Studies have confirmed that chloroform, tetrahydrofuran, ethyl acetate, dichloromethane, methyl-tert-butyl ether, and acetone can be safely and effectively used as mobile phases and sample diluents in the immobilization technology. Dimethylsulfoxide (DMSO) can be used with a slight loss of column efficiency. The column can be readily regenerated by flushing with DMF.

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Temperature Range

The narrow temperature range tolerated by coated polysaccharide CSPs mean temperature is rarely exploited as a means of controlling separation. The immobilized columns are stable to at least 80°C, giving an expanded temperature range which makes temperature a variable worth investigating.

The effect of temperature on chiral separations is fairly well established. In general, increasing temperature increases column efficiency but decreases both retention and enantioselectivity. The decrease in selectivity will vary between compounds, and the rate of decrease depends on the difference in binding enthalpies of the enantiomers. The effect of temperature on column efficiency depends on changes in mobile-phase viscosity, diffusion rates in the stationary phase, and kinetics of dissociation. Some separations are improved using sub-ambient temperature where the increased selectivity is sufficient to offset the loss in column efficiency.

Additives

Traditionally, diethylamine (DEA) is recommended as an amine additive for the analysis of basic compounds on polysaccharide phases. Studies conducted on CHIRALPAK IB have shown that ethylenediamine (EDA), ethanolamine (EtNA) and butylamine (BuA) are likely to enhance the resolution and peak shape of basic compounds separated on this column, when compared to the resolution obtained with DEA additive. Currently more investigations are needed to determine if these additives are equally as effective when used with the CHIRALPAK IA or IC columns.

CONCLUSION

A new, robust immobilization technology (Chiral Technologies' Platinum Series CHIRALPAK® columns) for chiral compound resolution provides, for the first-time, the ability to use virtually any organic solvent as a mobile phase or mobile phase component. The ability to use much wider variety of mobile phase solvents, temperatures, and solvents for sample dissolution opens up new possibilities for investigating conditions to accomplish separations that cannot be obtained with conventional columns. The indestructible qualities of the columns provide worry-free operation, eliminating the need to take extreme precautions to avoid forbidden solvents that damage or destroy conventional columns. An approach to method development for this new technology is provided here.



CHIRAL
TECHNOLOGIES INC

800 NORTH FIVE POINTS ROAD
WEST CHESTER, PA 19380 U.S.A.

1-800-6CHIRAL
1-610-594-2100
1-610-594-2325 FAX