CHIRALPAK Immobilized Columns

Q1. Are the CHIRALPAK IA, IB, and IC columns similar to any existing CHIRALPAK or CHIRALCEL® columns? How are the IA, IB, and IC columns different from coated polysaccharide columns?

A. The most important difference between the CHIRALPAK IA, IB, and IC columns and the traditional polysaccharide columns is their robustness and stability to mobile phase composition. In the CHIRALPAK IA, IB, and IC columns, the stationary phase is immobilized on the packing material instead of the coating process used in Daicel's other chiral columns.

This immobilization confers two major advantages. One is that the CSP can no longer be changed or destroyed by the use of a “forbidden” solvent — there are no forbidden organic solvents with the new columns. The other advantage is that with this total freedom of choice of solvent it is possible to develop new separations not previously possible, thus further extending the range of polysaccharide-based columns to chiral separations. See Question 7 for information on how the extended range of solvents can be used to attain high levels of success for method development with these new columns.

The similarities between CHIRALPAK IA and IB and the other columns lie in the chiral selector. Both columns are based on the same chiral selectors as CHIRALPAK AD-H and CHIRALCEL OD-H®: the 3,5-dimethylphenylcarbamate derivative of amylose and cellulose, respectively. CHIRALPAK IC, on the other hand, has a totally new chiral selector — the 3,5-dichlorophenylcarbamate derivative of cellulose — which is too soluble in common organic solvents to be available as a coated phase.

> The difference between CHIRALPAK IA / CHIRALPAK AD-H and between CHIRALPAK IB / CHIRALCEL OD-H lies in the immobilization of the stationary phase. Because the conformation of the polymer may be slightly influenced by the fact of the immobilization, there may be some small selectivity differences between the immobilized and coated support. These effects may be positive or negative, but are unimportant when compared to the far greater stability of the CSP to solvents, and to the possible improvement of separations through the exploitation of the wide range of solvents that may be used with these columns. As noted, CHIRALPAK IC has a unique chiral selector which is not available as a coated phase and therefore has the potential to develop unique separations not available with other Daicel CSPs.

Q2. How do CHIRALPAK IA, CHIRALPAK IB, and CHIRALPAK IC differ from one another?

A. CHIRALPAK IA, IB, and IC columns are generally complementary, much as are the four columns they nominally replace, namely CHIRALPAK AD-H, CHIRALCEL OD-H, CHIRALPAK AS-H, and CHIRALCEL OJ-H. Each column will separate a different set of enantiomeric compounds, although some compounds will resolve on more than one column. Generally, any compound that will separate on an AD-H column with a conventional mobile phase will separate on an IA column with the same mobile phase. Similarly, any compound that will separate on an OD-H column with a conventional mobile phase will generally separate on an IB column with the same mobile phase. In both cases

the retention may adjusted if necessary by a small solvent strength change. The CHIRALPAK IC column has some overlap with the other two columns but also displays some unique selectivity. In combination, all three columns have been shown to allow development of chiral separations for most racemates when both conventional and extended range mobile phases are used.

Q3. What solvents can I use with CHIRALPAK IA, IB, and IC columns? Are there any solvents that I should not use with the immobilized columns?

A. There are currently no known organic solvents which will damage the CHIRALPAK IA, IB, or IC columns in any way.

Extensive testing has been carried out with many common organic solvents, including hexane or heptane/alcohols, methanol, isopropanol, ethanol, acetonitrile, dichloromethane, chloroform, tetrahydrofuran, ethyl acetate, acetone, methyl acetate, MTBE, dimethylformamide, dimethylacetamide, etc.

Where the columns have been extensively used and have perhaps become fouled with impurities or non-eluted compounds they can readily be cleaned by flushing with dimethylformamide, THF, or ethyl acetate.

When used in reversed-phase chromatography, the columns should not be operated below pH 2 or above pH 7. The upper range of the CHIRALPAK IA column only can be extended to pH 9, provided that borate buffer is employed, and that a guard cartridge is used and is changed at least once every 200 injections at this pH.

Q4. When using the CHIRALPAK IA, IB, or IC columns, is there any problem with diluting my sample in a solvent other than mobile phase?

A. Generally, the best procedure is to dissolve a sample in mobile phase, whenever possible. Care must still be taken when injecting sample dissolved in a solvent that has greater solvating power than mobile phase. This is a general chromatographic problem in that this may cause distortion of the chromatographic peaks, thus affecting the analytical results. In preparative chromatography there is a risk that sample from a concentrated injection in a good solvent will precipitate on the column, once the sample comes in contact with a mobile phase in which it has poorer solubility. An additional problem could occur if the column has greater attraction for sample diluent than for mobile phase. In such cases, sample diluent may stick to the column and affect the selectivity or efficiency of future injections.

For some applications, samples are presented in dimethylsulfoxide (DMSO). This solvent will not permanently harm these chiral stationary phases but is retained on the column under certain mobile phase conditions. Repeated injections of samples diluted in DMSO may produce a gradual decrease in column efficiency on a CHIRALPAK IA column which can be corrected by occasional flushing with dimethylformamide. Injections of samples diluted in DMSO are not recommended on CHIRALPAK IB columns, as such injections cause a more severe and immediate decline in column efficiency due to retention of this

solvent on the column. This too is reversible following flushing of the column to remove the DMSO.

Of course, since the stationary phase is immobilized, there are no issues with stability of the column under any of the above circumstances.

Q5. What are the advantages of the new CHIRALPAK IA, IB, and IC columns? Should I choose a coated or immobilized column for my application?

A. Unless there are special circumstances, it is strongly recommended that immobilized columns be used for any application. This is because of their far greater stability in operation than other Daicel chiral columns. Another advantage of immobilized columns is their stability to strong solvents like THF, ethyl acetate, and the chlorinated solvents. This allows development of separations in these solvents which often give different selectivity in comparison with the usual solvent set used for chiral chromatography. Further, in preparative chromatographic applications, the use of such solvents can greatly enhance the sample solubility and thus the potential production rate for the separation.

In circumstances where a conventional, coated column is specified (in a validated procedure where an immobilized column is not yet approved, or when an existing method meets the specs of the separation) it may be appropriate to use coated columns rather than immobilized columns.

During the introduction of the full range of immobilized columns, there may be separations requiring a selectivity for which there is not yet an immobilized column. In such cases, other Daicel columns remain available for use.

Q6. Are there any different mobile phase modifiers or additives for the CHIRALPAK IA, IB, and IC columns?

A. The same additives — diethylamine for basic compounds and trifluoroacetic acid for acidic compounds — used for other chiral columns may be used with CHIRALPAK IA, IB, and IC. Studies conducted on the CHIRALPAK IB column have shown that ethylenediamine (EDA), ethanolamine (EtNA), and butylamine (BuA) may enhance the resolution and peak shape of basic compounds separated on that column compared to the resolution obtained with DEA additive.

A good rule of thumb is to use a basic additive with an amine functionality similar to that of the compound being resolved

Q7. How do I develop separation methods using the range of Daicel's immobilized chiral columns?

A. Using the immobilized columns allows a greater freedom of solvent choice than for the coated columns. Since it is not possible to predict the selectivity of polysaccharide-

based chiral phases from knowledge of the structure of the solutes, conventionally a screening process is followed. After studying a very wide range of solvent possibilities, we have found a set of four solvent types that allow a high success rate in the initial screening that reduces the time required for the initial stages of method development.

The table 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 the one of the screening mobile phases. 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 come out too quickly then 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.

Table: 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.

Usually, one of the experiments performed in the initial screening will give a strong indication of the direction to follow for subsequent work. If alternative solvents turn out to be necessary to improve the selectivity further, we have found the following possibilities to be useful:

Extended Range			Polar Mode	
Hexane/Ethyl Acetate 70:30	Hexane: Chloroform: Ethanol 65:30:5	Hexane: Tetrahydrofuran 70:30	Methanol or Methanol: Ethanol 50:50	Acetonitrile 100

Again, it should be noted that the Extended Range solvents will destroy coated polysaccharide-based columns.