1 - Which column would be the most appropriate for my application ?

Due to the complexity of the chiral recognition mechanism, it is not possible yet to establish rules for the selection of the best chiral stationary phase for any given compound.
The right column is normally found by a combination of experience and experimentation.

1.1 Literature search

Often, but not always, similar compounds can be resolved using the same conditions. A first step consisting of a literature search will determine if a given molecule (or a similar one) has already been separated using a particular column.
Chiral Technologies Europe can assist you with this literature search. Following receipt of the compound structure (or at least some specific derivatives) a search based on structural similarities can be performed.
As a result of this search, we can usually recommend a column that is more likely to separate the compound of interest.

1.2 Other tools available

If the literature search fails to provide a recommended column, a statistical program, Chiral Tool, is available from Chiral Technologies Europe. The aim of Chiral Tool is to provide statistical information about the chemical features associated with compounds analysed using DAICEL columns. This program can be of help to select a column, but is not a stand-alone enantioselectivity prediction system. A detailed description of the Chiral Tool concept as well as guidelines to run the program are provided in the Help session.

DAICEL's Application guide for column selection is also accessible on the internet at http://www.daicel.co.jp/chiral/application/application.html. This application guide lists a selection of over 350 non-proprietary racemic compounds that have been successfully separated using DAICEL columns.

1.3 Our experience

A statistical evaluation of a large number of compounds indicates that 50 to 60% of all racemic samples analysed in our application laboratories can be separated on the CHIRALCEL® OD-H and the CHIRALPAK® AD-H columns with the CHIRALPAK® AD-H being a little more universal compared to the CHIRALCEL® OD-H. An additional 20% of compounds are separated on the CHIRALPAK® AS-H and the CHIRALCEL® OJ-H columns. These 4 columns will offer you the best chance to achieve the chiral separation of your molecules, tried in the following order:

CHIRALPAK® AD-H > CHIRALCEL® OD-H > CHIRALCEL® OJ-H # CHIRALPAK® AS-H

1.4 Experimental screening

Chiral Technologies' activities include the method development dedicated to analytical, semi-preparative and preparative applications.
By performing a complete screening using all available DAICEL chiral stationary phases, the best conditions will be identified to meet our customers' requirements. For more detailed information, you can send an e-mail to support@chiral.fr.

2 - Which solvents are compatible with my DAICEL column ?

Many of the solvents commonly used in HPLC eluents such as acetone, chloroform, DMF, dimethylsulfoxide, toluene, ethyl acetate, methylene chloride and THF may DESTROY the chiral stationary phase.
Please, be aware that even small quantities of incompatible solvents introduced in sample dilutions for instance, or left in the transfer lines (including autosampler lines) can affect rapidly and irreversibly the column performance.
Even if present in residual quantities, such solvents are likely to shorten the life of a column.

By taking a few simple precautions, you can significantly enhance the lifetime of DAICEL columns. Always read the instruction manual shipped with each column before exposing your column to any mobile phase. Each type of column has a specific instruction manual that refers to operating conditions that can be used only with that column.

By taking a few simple precautions, you can significantly enhance the lifetime of DAICEL columns. Always read the instruction manual shipped with each column before exposing your column to any mobile phase. Each type of column has a specific instruction manual that refers to operating conditions that can be used only with that column.
Standard conditions for DAICEL polysaccharide-type columns are alkane/alcohol solvent mixtures. However, polar solvents like ethanol, methanol or acetonitrile are tolerated by some of them. As the use of such solvents requires cautious handling, you are welcome to contact us for further assistance (support@chiral.fr) and to read carefully the instruction sheet shipped together with your column.

In addition, the reversed phase versions of our four most versatile columns (CHIRALPAK® AD-RH, AS-RH and CHIRALCEL® OD-RH, OJ-RH) have been developed for samples that require aqueous mobile phase (biological samples for instance). Suitable operating conditions for these reversed phase columns are fairly well detailed in the instruction manuals.

All the instruction manuals are available on our website as well as a table of compatible solvents for our most popular columns.

3 - Why is it sometimes necessary to use additives (modifiers) in the mobile phase ?

Strongly basic or strongly acidic compounds will tend to adsorb onto the chiral stationary phase on active sites of the silica support, resulting in broad and/or tailing peaks observed. In order to remove or to minimize these undesired interactions, additives are added to the mobile phase. These additives will preferentially adsorb on the most active sites, displacing solute molecules and making these sites unavailable for solute adsorption.

Additives most frequently used are:

Trifluoroacetic acid (TFA) for acidic compounds
N,N-Diethylamine (DEA) for basic compounds

Important note: other acids like acetic or formic acid, and other bases like ethanolamine or butanolamine can also be used.
If non-optimal peak shapes are still observed with TFA or DEA, the effect of these other additives should be tried.

All these additives can be added to the mobile phase at concentrations up to 0.5%. But in most cases, 0.1% is sufficient. Longer column life may result if the concentration can be kept to 0.1%-0.2%.

A second advantage of these additives is that they can enhance the solubility of compounds that would otherwise have a low solubility in a given mobile phase.

In the case of the reversed phase columns (CHIRALCEL® OD-R, OD-RH, OJ-RH and CHIRALPAK® AD-RH, AS-RH), the control of mobile phase pH is more important than the actual level of additive employed. These columns are stable in the pH range from 2.0 to 7.0 and can be used in the extended pH range of 7.0-9.0 with borate buffer under special conditions (refer to the respective instruction sheets).

pH 2.0 phosphate buffer and pH 9.0 borate buffer are the recommended starting conditions for acidic and basic solutes respectively that are likely to require additives.

4 - My column is loosing its performance. Is there a regeneration procedure ?

All DAICEL columns have been tested before packaging. Test parameters and results are shipped together with each column. The column should be evaluated regularly under the same conditions as the original test chromatogram to properly follow-up on its performance.

4.1 Column performance declines rapidly:

In most cases it is the result of a column unintentionally exposed to some harmful substance. To prevent such an occurrence, it is absolutely necessary to ensure that the entire HPLC system including both the injector and the injection loop, has been flushed with a solvent compatible with the column and its storage solvent prior to connection.
Proper sample clean-up and preparation are also vital. Small amounts of non-allowed solvent in a sample preparation may seem insignificant, but these low level residues often dissolve column packing, which leads to a rapid decay in the column performance (refer to Question 2).
It is usually not possible to recover the column performance by washing or regeneration if columns have lost a significant amount of stationary phase.

4.2 Column performance declines progressively

This is most often the result of material adsorbing onto the chiral stationary phase. In this case, it may be possible to regenerate the column by following the washing procedure that can be found on the column instruction sheet.
If recommended washing fails to restore performance, more drastic washing or changing the column frits may be needed.
Please contact support@chiral.fr for more information on such operations that can carry a significant risk to damage the column.

4.3 Problems in reproducing analyses

It may happen that an established separation cannot be duplicated on a new column. This situation is often caused by some "memory effect" an older column may have, due to the use of additives which have been employed in the past column history. As these additives adsorb onto the stationary phase and are crucial to a given separation, the problem can be resolved by conditioning the new column for a few hours with the mobile phase that contains the pertinent additive (refer to Question 3).

In those cases in which the separation cannot be restored (small batch-to-batch variations in column performance may always be possible), the method may possibly be redeveloped (different operating conditions and/or column).

5 - My sample is not soluble in alkane/alcohol solvent mixtures. Which solvent can I use?

In the best interest of the reproducibility of the analyses, it is highly recommended to prepare the sample in the mobile phase. For analytical purposes, high sample concentrations are usually not necessary. A sample preparation of about 0.5 to 1mg/ml or even less in the mobile phase is often sufficient. For acidic compounds or basic salts, the additive contained in the mobile phase may improve the solubility (refer to Question 3).

If your sample still doesn't have good solubility, then try to dissolve it in 100% alcohol (methanol, ethanol, 2-propanol). Avoid using acetonitrile when a mobile phase containing alkane is used.

Forbidden solvents include:
acetone, chloroform, toluene, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, MEK, MTBE, THF and pyridine.

Even small amounts of these solvents may DESTROY the chiral stationary phase.

For samples that only dissolve in aqueous media, it would be then more appropriated to work in reversed phase mode. Mobile phase and dilution solvents of alcohol/water or acetonitrile/water can be used with the CHIRALCEL® OD-R, OD-RH, OJ-RH and the CHIRALPAK® AD-RH and AS-RH reversed phase columns.

6 - My DAICEL column has a high back-pressure value

A high pressure in the HPLC system probably indicates that there is a partial blockage of: - the LC system itself, or
- the column.
If the system pressure free of the column is abnormally high under normal operating conditions, the blockage should be located (a connecting tubing, an in-line filter or valve channel), and the faulty component replaced.
If the column has been identified to be responsible for the high back-pressure observed, it is very often caused by impurities blocking the frits.
In some cases, these impurities may be removed by performing the recommended washing procedures. To prevent such problems, it is always wise to use a replaceable in-line filter or a guard column before the column, and also to filter the samples prior to injection.

The back pressure value also depends on the mobile phase viscosity. Due to the high viscosity of solvents such as ethanol or 2-propanol, a high back pressure value is observed at normal flow rates. In this case, the flow rate has to be adjusted (reduced) until the pressure falls below the recommended upper limit.
For reversed phase columns (-RH), the high viscosity of the aqueous mobile phases + the small particle size require an operating flow rate of 0.5ml/min or less.

When semi-preparative columns are operated, you may need to increase either the diameter of the connecting tubing or the volume of the detector flow cell in order to work at the highest flow rate calculated from the analytical column (linear scale-up). Alternatively, the flow rate applied to the semi-preparative column needs to be reduced for the pressure to remain below the recommended upper limit.

Wrong operating conditions (introduction of incompatible solvent from samples or mobile

7 - Do I need a guard column ?

The purpose of a guard column is to protect the analytical or semi-preparative column from materials that would either adsorb on the stationary phase, or would dissolve some of the packing material.

Guard columns has a sacrificial role: when a guard column is nearing the failure point, it can easily be replaced at a fraction of the cost of a new column. Loss in separations, increased peak broadening or tailing, increased pressure drop in your system, are all signals that indicate a guard column may need to be replaced.

The guard column should contain the same packing material as the analytical or semi-preparative column. Specific guard columns are available for all DAICEL columns. A non-specific guard column might adsorb some impurities from the sample or the mobile phase; however, it may diminish the separation.

Detailed information regarding guard columns and guard cartridges are mentioned in our catalogue.

8 - Can I use my column in SFC ?

SFC works very well with DAICEL Chiral columns. Carbon dioxide as a mobile phase bulk fluid has solvent properties similar to hexane with lower viscosity and flammability.

Carbon dioxide can be used with all the solvents which are compatible with DAICEL columns (alcohols and acetonitrile). Chiral selectivity is normally comparable in SFC and HPLC but better resolution is observed in SFC due to its higher efficiency at typical flow rates. Higher flow rates may be used in SFC because of the low viscosity of CO2 resulting in faster separations. We have discovered that carboxylic acids, requiring acidic mobile phase additives in HPLC, can be eluted in SFC without such additives.

A common concern is the effect of the high pressures used in SFC on column stability. The pressure drop across the column is the important factor in column stability. This pressure drop is lower in SFC than HPLC, and DAICEL columns have proved to be very stable under SFC conditions.

We recommend that when the column is not in use, the CO2 should be displaced by flushing briefly with 2-propanol.
When using a DAICEL column in SFC that had been used in HPLC, it is necessary to first flush the hexane with 2-propanol, as CO2 will not efficiently flush hexane, and a noisy baseline will result.

SFC can offer several advantages in preparative applications. Separations are faster, so is the isolation of the product from the mobile phase as the bulk of the mobile phase evaporates as part of the collection process. With the lower pressure drop experienced in SFC, the use of higher efficiency 5 micron particle H-series columns for preparative application is feasible. Chiral Technologies offers 1, 2 and 3 cm ID columns packed with AD-H, AS-H, OD-H or OJ-H in specific SFC column hardware.

9 - I need basic guidelines to properly handle my column

> Flush the entire HPLC system including the injector, the sample loop and the detector with a solvent compatible with the column and its storage solvent (with 2-propanol for instance) before connecting the column.

> Only use solvents which are listed in the column instruction manual to ensure maximum column life. Do not hesitate to take advice at support@chiral.fr if you need more assistance for mobile phase preparation.
Not all columns are compatible with the same solvents. Each column is delivered with its specific instructions sheet. If you lose the instruction sheet of your column, we can easily replace it, or you can consult our website.

> Use simple mobile phases: separations on normal phase columns are usually achieved with simple mobile phase such as alkane/alcohol mixtures.
Note that several polysaccharide stationary phases require some limitations in the ratio of alkane/alcohols (see individual instruction sheets provided with each column)100% Alcohols or acetonitrile are used to operate columns under polar organic mode. These conditions are not suitable for all DAICEL columns (see individual instruction sheets provided with each column). Optimal results, with minimal risk of column damage are obtained with columns specifically dedicated to polar organic mode.
Do not hesitate to contact support@chiral.fr before trying such conditions with your columns.

All solvents should be HPLC grade.

> Remember that depending on the nature of the compound to analyse, a basic or an acidic additive should be added to the mobile phase (refer to Question 3).

> Equilibrate the column to a stable baseline. A minimum of 30 minutes at a flow rate of 1ml/min is usually required.

> Samples should be dissolved in mobile phase constituents only, to avoid on-column precipitation and/or injected solvent effects.
Samples should be free of insoluble particles. It is recommended to filter all samples or to use an in-line filter.
To prevent contamination of the main column, a guard column should always be installed upstream of the column.

> Flush the column with the appropriate storage solvent when analyses are completed. Very often, acidic or basic modifiers like TFA or DEA are added to the mobile phase. If the column is not used for more than several days, these additives have to be removed by flushing the column with the mobile phase that does not contain any acid or base.
For the reversed phase columns commonly used with buffer solutions, any traces of salts should be removed by flushing thoroughly with the mobile phase that does not contain salt/buffer before converting to the recommended storage conditions.
When a column is no longer being used, it should be removed from the HPLC system and tightly capped at both ends to avoid solvent evaporation.

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