CHIRAL HPLC METHOD DEVELOPMENT

CHOOSING A COLUMN

Chiral Screening Column Sets

We recommend building a small library of chiral columns that have broad applicability and durability for high volume screening. We can suggest a column set that matches your needs and covers the broadest spectrum of selectivity.

Suggested columns in order of preference:

- WHELK-O® 1
- REFLECT™ I-Amylose A
- REFLECT™ I-Cellulose B
- REFLECT™ I-Cellulose C
- REFLECT™ I-Cellulose J
- REFLECT™ I-Cellulose Z
- REFLECT™ C-Amylose A
- REFLECT™ C-Cellulose B
- Other Pirkle-type phases
- *ULMO, DACH-DNB, etc.*

REFLECT™ Polysaccharide Chiral Columns

Most popular type of CSP, suitable for a wide range of chiral compounds.

Column	Selector Type	Equivalent Competitive Column
Coated Polysaccharide Phases		
REFLECT™ C-Amylose A	Amylose tris (3,5-dimethylphenylcarbamate)	CHIRALPAK® AD, AD-H, AD-3; LUX® Amylose-1
REFLECT™ C-Cellulose B	Cellulose tris (3,5-dimethylphenylcarbamate)	CHIRALCEL® OD, OD-H, OD-3; LUX® Cellulose-1
Immobilized Polysaccharide Phases		
REFLECT™ I-Amylose A	Amylose tris (3,5-dimethylphenylcarbamate)	CHIRALPAK® IA, IA-3; LUX® i-Amylose 1
REFLECT™ I-Cellulose B	Cellulose tris (3,5-dimethylphenylcarbamate)	CHIRALPAK® IB, IB-3
REFLECT™ I-Cellulose C	Cellulose tris (3,5-dichlorophenylcarbamate)	CHIRALPAK® IC, IC-3; LUX® i-Cellulose 5
REFLECT™ I-Cellulose J	Cellulose tris (4-methylbenzoate)	CHIRALPAK® IJ, IJ-3
REFLECT™ I-Cellulose Z	Cellulose tris (3-chloro-4-methylphenylcarbamate)	Similar to CHIRALCEL® OZ; LUX® Cellulose-2

Pirkle-type Chiral Columns

Broad applicability to a wide range of compounds, and alternative selectivity to polysaccharide chiral columns.

The most popular Pirkle-type phase, WHELK-O® 1, is available in a full range of particle sizes from 1.8 - 20 µm to serve small scale analytical separations up to large scale preparative work. These columns are available in both R,R and S,S configurations, allowing for peak elution order to be inverted. This can be beneficial in preparative and process scale applications when needing to elute one peak of interest quickly.

π -Electron Acceptor/ π-Electron Donor Phases

- WHELK-O® 1
- WHELK-O® 2
- ULMO

π-Electron Acceptor Phases

- DACH-DNB
- Pirkle 1-J
- α-Burke 2
- β-Gem 1
- Leucine
- Phenylglycine

CHIROSIL® Crown Ether Chiral Columns

CHIROSIL® columns are ideal for the separation of chiral compounds containing primary amines and natural and unnatural amino acids and their derivatives.

CHOOSE A COLUMN

→ **CHOOSE A MOBILE PHASE AND ADDITIVE**

Sample Elutes in the Void → **Decrease the Strong Solvent by Half**

- **Sample is Out of the Void with Poor Peak Shape** → **CHOOSE A DIFFERENT STRONG SOLVENT, ADDITIVE, OR COLUMN**
- **Sample is Out of the Void with No Resolution** → **CHOOSE A DIFFERENT STRONG SOLVENT, ADDITIVE, OR COLUMN**
- **Sample is Out of the Void with Good Resolution** → **OPTIMIZE THE METHOD**

Adequate Retention

- **Poor Resolution** → **CHOOSE A DIFFERENT STRONG SOLVENT, ADDITIVE, OR COLUMN**
- **Acceptable Resolution** → **OPTIMIZE THE METHOD**

OPTIMIZE THE METHOD

- Solvent Composition
- Flow Rate
- Column Dimensions
- Particle Size
- Change Mobile Phase Additive

TIP

We recommend dedicating columns to either reversed-phase or normal phase operation. When switching a column from normal phase to reversed-phase (or vise versa), be sure to transition the column through a solvent that is miscible with both mobile phase systems.

Choosing a Mobile Phase

- Factors such as solubility and future considerations for preparative work usually help determine whether to develop methods with normal phase or reversed-phase solvents.
- Pirkle-type phases can be used in either mode, but typically perform best with normal phase solvents.
- Since many analytical chiral methods later scale up to preparative separations, we recommend using normal phase first.
- We also recommend the starting mobile phase should be the strongest solvent combination that allows full sample solubility.

Choosing a Strong Solvent

Normal phase separations

Start with mobile phase of 50/50 alkane/alcohol. A strong solvent will help to elute peaks quickly.

- Alkane: hexane, heptane
- Alcohol: ethanol, isopropyl alcohol

For immobilized polysaccharide and Pirkle-type columns, you can try additional mobile phases including:

- Alkane/dichloromethane
- Dichloromethane/alcohol
- Methyl tert-butyl ether/alcohol

Note: *Do Not* use these mobile phases with coated polysaccharide columns

Reversed-phase separations

Start with a mobile phase of 50/50 water/organic.

- Organic: acetonitrile, methanol

Choosing a Mobile Phase Additive

Depending on the nature of the sample, a mobile phase additive is often needed in order to achieve good peak shape and improve resolution.

Normal phase separations

- Acidic samples: we recommend using 0.1% acid (e.g. acetic acid or TFA).
- Basic samples: we recommend using 0.1% base (e.g. triethylamine or diethylamine).

Reversed phase separations

- Acidic samples: start with 0.1% formic acid in the aqueous component.
- Basic samples: start with 20 mM ammonium bicarbonate in the aqueous component.

Optimizing Your Method

Many approaches can be taken to optimize chiral methods:

- Decreasing mobile phase strength increases retention and often improves peak resolution.
- Changing the strong solvent can change selectivity and peak resolution.
- The mode of chromatography can be changed.
- Column dimensions and particle size can be changed to accommodate needs.

We suggest you keep it as simple as possible. Once you have achieved an acceptable separation, move on to the next project. Small increases in resolution and alpha are usually not worth the time spent in method development to achieve those increases.

FREE Chiral Screening Service

Let REGIS find the right column and method for your compound. Results in as little as 3 days.

Get started today!

registech.com/chiral-screening/

Chiral Applications Database

Browse our library of over 950 chiral applications using HPLC and SFC techniques.

Visit to learn more.

registech.com/chiral-applications/

Chiral Handbook

The Chiral Handbook provides HPLC and SFC method development advice and contains more than 950 chiral applications.

Download or request a copy.

registech.com/request-chiral-handbook-for-hplc-sfc-separations/

Product & Technical Support

Contact our experienced chromatography experts for product information or technical support.

Contact REGIS today!

Product Info: **chromsales@registech.com**
Tech Support: **techsupport@registech.com**

RECOMMENDATIONS

Maximum Column Operating Pressures

Particle Size (µm)	Length (mm)	I.D. (mm)	Pressure (psi)
1.8	50, 100	2.1, 3.0, 4.6	12,000
2.7, 3	50, 100, 150, 250	2.1, 3.0, 4.6	8,000
5, 10	50, 100, 150, 250	2.1, 3.0, 4.6	6,000
5, 10	100, 150, 250	10, 21.1	5,000
5, 10	100, 150, 250, 500	30, 50	3,000

Scaling Flow Rates (mL/min) based on d_p I.D.

Column I.D. (mm)	Particle Size (µm) 1.8	3.5	5.0	10.0	16.0	20.0
2.1	0.87	0.45	0.31	0.16	0.10	0.08
3.0	1.8	0.91	0.64	0.32	0.20	0.16
4.6	4.6	2.1	1.5	0.75	0.47	0.37
10.0	20	10	7.1	3.5	2.2	1.8
21.1	88	45	32	16	10	8
30.0	177	91	64	32	20	16
50.0	492	253	177	88	55	44

Use this chart when changing column i.d. and/or particle size to estimate the new optimal flow rate. Values shown are based on normal phase conditions.

Column Solvent Compatibility

Column Family	Solvent Restrictions
WHELK-O® 1 and other Pirkle-type Phases	None
REFLECT™ Immobilized	None
REFLECT™ Coated and other Coated Polysaccharide Phases	Do not use: • Acetone • Chloroform • DMF • DMSO • Methylene Chloride • THF Up to 90% water OK Acid and base modifiers should not exceed 0.5%
CHIROSIL® Phases	None

Estimated Sample Loading

Type of Column	Dimensions	Packing Material	Sample Loading
Analytical	250 x 4.6 mm	~3.5 grams	14 - 56 mg/injection
Semi-Prep	250 x 10.0 mm	~16 grams	64 - 256 mg/injection
Prep	250 x 21.1 mm	~72.5 grams	288 - 1,152 mg/injection

Sample loading is most affected by sample solubility in the mobile phase.

chiral.com

MZ ANALYSENTECHNIK

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e-mail: info@mz-at.de, www.mz-at.de

REGIS® TECHNOLOGIES, INC.

CHIRAL SFC METHOD DEVELOPMENT

CHOOSING A COLUMN

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We recommend building a small library of chiral columns that have broad applicability and durability for high volume screening. We can suggest a column set that matches your needs and covers the broadest spectrum of selectivity.

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- REFLECT™ I-Cellulose J
- REFLECT™ I-Cellulose Z
- REFLECT™ C-Amylose A
- REFLECT™ C-Cellulose B
- Other Pirkle-type phases
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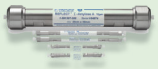

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REFLECT™ I-Cellulose C	Cellulose tris (3,5-dichlorophenylcarbamate)	CHIRALPAK® IC, IC-3; LUX® I-Cellulose 5
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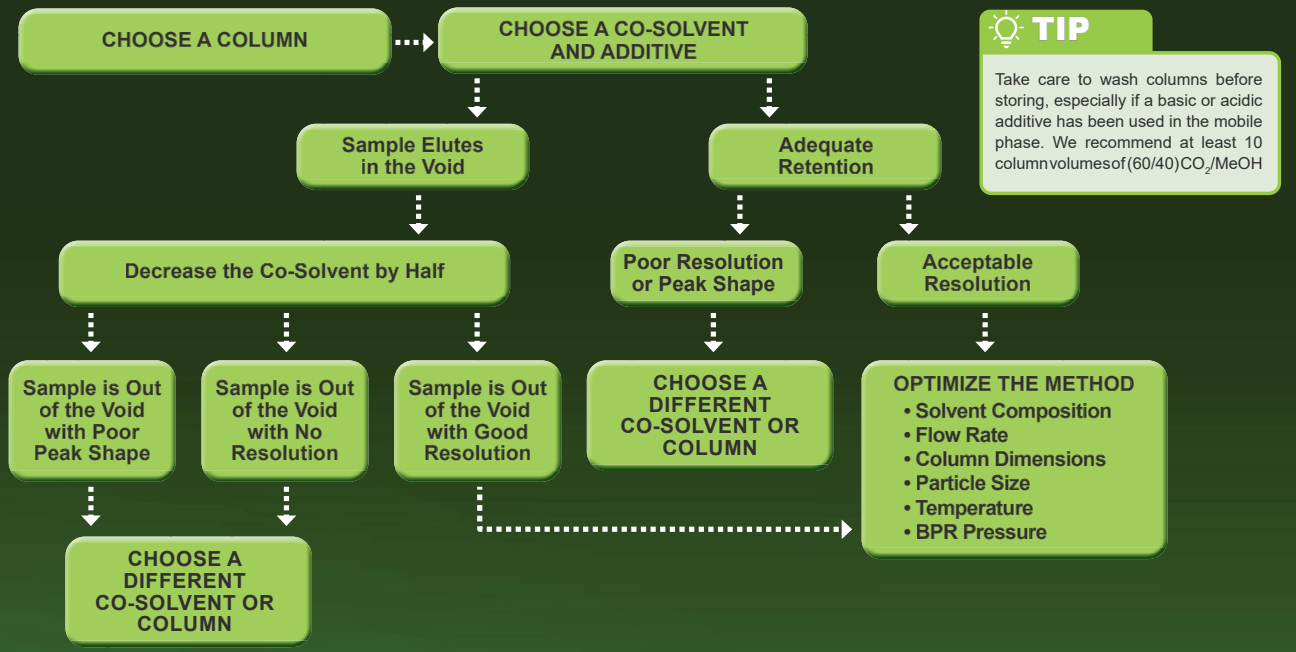

TIP

Take care to wash columns before storing, especially if a basic or acidic additive has been used in the mobile phase. We recommend at least 10 column volumes of (60/40) CO_2/MeOH

Choosing an SFC Mobile Phase Co-Solvent

Supercritical CO_2 is the major mobile phase component in SFC separations. Its low viscosity and high diffusivity allows for very fast, high-efficiency separations. Because of CO_2's low polarity, however, it is often necessary to add a polar co-solvent to elute analytes. Alcoholic co-solvents, like Methanol, Ethanol, or Isopropanol are common. For separations involving low-polarity analytes, acetonitrile is a good alternative to alcoholic co-solvents.

Ternary mobile-phases (e.g. CO_2/MeOH/IPA) can be investigated to balance retention and resolution considerations. They may also be useful when separating analytes with multiple chiral centers.

Immobilized polysaccharide phases and Pirkle phases expand the range of co-solvents that can be explored during screening or method development.

The following co-solvents can be used, except with coated polysaccharide phases.

- Dichloromethane: either alone or as a ternary solvent with an alcohol
- Ethyl acetate • Methyl tert-butyl ether

Choosing a Mobile Phase Additive

Depending on the nature of the sample, a mobile phase additive is often needed in order to achieve good peak shape and improve resolution.

CO_2/MeOH mobile phases have been found to be slightly acidic due to the formation of methyl carbonic acid.

- For acidic compounds, it may still be necessary to add an acid additive to the co-solvent.
- We recommend using 0.1 - 0.5% acid (e.g. acetic acid, TFA) in the co-solvent.
- For basic compounds, 0.1 - 0.5% base (e.g. DEA, TEA, etc.) can be added to the co-solvent.

Recent studies have highlighted the addition of up to 10% water in methanol to separate very polar analytes.

Optimizing Your Method

Many approaches can be taken to optimize chiral methods:

- Decreasing mobile phase strength increases retention and often improves peak resolution.
- Changing the co-solvent can change selectivity and peak resolution.
- Column dimensions and particle size can be changed to accommodate needs and capabilities.

We suggest you keep it as simple as possible. Once you have achieved an acceptable separation, move on to the next project. Small increases in resolution and alpha are usually not worth the time spent in method development to achieve those increases.

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Scaling Flow Rates (mL/min) based on d_p I.D.

Column I.D. (mm)	Particle Size (µm) 1.8	3.5	5.0	10.0	16.0	20.0
2.1	2.3	1.2	0.83	0.42	0.26	0.21
3.0	4.7	2.4	1.7	0.85	0.53	0.43
4.6	11	5.7	4.0	2.0	1.2	1.0
10.0	53	27	19	9.4	5.9	4.7
21.1	230	120	84	42	26	21
30.0	470	240	170	85	53	43
50.0	1300	680	470	240	150	118

Use this chart when changing column i.d. and/or particle size to estimate the new optimal flow rate. Values shown are based on normal phase conditions.

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Chiral Applications Database

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Chiral Handbook

The Chiral Handbook provides HPLC and SFC method development advice and contains more than 950 chiral applications.

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registech.com/request-chiral-handbook-for-hplc-sfc-separations/

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Contact REGIS today!

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