



Chiral HPLC Column Selection and Method Development Guide

Choosing a Column Based on Application Area

Chiral method development begins with choosing the right columns to screen. Some suggested effective combinations can be found in the Chiral Screening Kits below; they are especially useful when finding a method for a novel compound. The wide range of phases offered by Supelco covers most application areas, as shown in the table below. For further assistance in choosing a column, please call our Tech Service, or consult our online application library and bibliography. Once you've narrowed down your choice of column, you can use this guide to help you develop and optimize your method.

Application/Field of Use	CHIRAL PHASE							
	Astec Cellulose DMP & Kromasil® Cellucoat™ <small>DMP: derivatized cellulose</small>	Astec CHIROBIOTIC® <small>Macrocytic glycopeptides</small>	Astec CYCLOBOND® <small>Native & derivatized cyclodextrins</small>	Astec P-CAP™ & P-CAP-DP <small>Synthetic chiral polymers</small>	Astec CLC <small>Copper ligand exchange</small>	CHIRAL-AGP, HSA, CBH <small>Immobilized proteins</small>	LARIHC™ <small>Derivatized cyclodextrins</small>	Astec CHIRALDEX® & Supelco DEX™ <small>Derivatized cyclodextrins for GC</small>
Routine Chiral Column Screening	■	■	■	■	■	■	■	■
Normal Phase HPLC	■	■	■	■	■	■	■	■
SFC	■	■	■	■	■	■	■	■
Reversed-Phase HPLC	■	■	■	■	■	■	■	■
Hydrophilic Interaction HPLC (HILIC)	■	■	■	■	■	■	■	■
Polar Organic Mode (HPLC)	■	■	■	■	■	■	■	■
Polar Ionic Mode (HPLC)	■	■	■	■	■	■	■	■
Ligand Exchange Mode HPLC	■	■	■	■	■	■	■	■
Gas Chromatography (GC)	■	■	■	■	■	■	■	■
Prep (LC and/or SFC)	■	■	■	■	■	■	■	■
Polar/Ionic Analytes	■	■	■	■	■	■	■	■
Amino Acids, Peptides	■	■	■	■	■	■	■	■
Primary Amines, Chiral	■	■	■	■	■	■	■	■
Non-Aromatic Organic Acids	■	■	■	■	■	■	■	■
Mass Spec (LC/ESI)	■	■	■	■	■	■	■	■
Mass Spec (LC/APCI)	■	■	■	■	■	■	■	■
Mass Spec (GC/MS)	■	■	■	■	■	■	■	■
Bioanalysis (drugs in biological fluids)	■	■	■	■	■	■	■	■

■ Highly suitable ■ Partially suitable, or limited to specific applications ■ Not suitable or recommended

Choosing a Column Based on CSP Type

The chiral selectors of today's successful CSPs are based on or mimic complex biomolecules, like proteins, peptides, and carbohydrates. The abundance of types of CSPs is necessary: each enantiomer separation is unique and requires specific differentiating interactions. Choosing a CSP is often based on the preferred mobile phase system, for optimal sample solubility, preparative considerations, or instrument compatibility.

Choice of Chiral HPLC and SFC Phases from Supelco

Type of CSP	Chiral Selectors (Phases)	Product Line
Polysaccharide	tris-(3,5-Dimethylphenyl) carbamoyl cellulose tris-(3,5-Dimethylphenyl) carbamoyl amylose	Astec Cellulose DMP, Kromasil CelluCoat Kromasil® AmyCoat™
Macrocytic glycopeptide	vancomycin, teicoplanin, teicoplanin aglycone, ristocetin A	Astec CHIROBIOTIC®
Cyclodextrin	β- and γ-cyclodextrins, native and derivatized	Astec CYCLOBOND®
Protein	α-acid glycoprotein, cellobiohydrolase, human serum albumin	CHIRAL-AGP, CHIRAL-CBH, CHIRAL-HSA
Chiral synthetic polymer	poly(trans-1,2-cyclohexanedyl-bis-acrylamide) poly(diphenylethylenediamine-bis-acryloyl) O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-L-tartar diamide O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide	Astec P-CAP™ Astec P-CAP™-DP Kromasil® Chiral DMB Kromasil® Chiral TBB
Chiral ligand exchange	chiral bidentate ligand	Astec CLC-L, Astec CLC-D
Cyclodextrin	derivatized cyclodextran 6, cyclodextran 7	LARIHC™

Chiral Column Screening Kits

Our chiral column screening kits provide the necessary columns to perform most chiral separations and run mechanistic studies, and are offered at very attractive prices.

HPLC Column Screening Kit*

Cat. No.	Description
	Astec CHIROBIOTIC Column Screening Kit Contains one each Astec CHIROBIOTIC V2, T, R, and TAG
10300AST	10 cm x 4.6 mm I.D., 5 μm
10305AST	25 cm x 4.6 mm I.D., 5 μm
	Astec CYCLOBOND Column Screening Kit Contains one each Astec CYCLOBOND 1 2000, CYCLOBOND 1 2000 DMP, CYCLOBOND 1 2000 HP-RSP, and CYCLOBOND 1 2000 DNP
20005AST	25 cm x 4.6 mm I.D., 5 μm

GC Column Screening Kit*

Cat. No.	Description
	Astec CHIRALDEX Column Screening Kit – broadest range of applications Contains one each Astec CHIRALDEX G-TA, B-DM, and B-DA
71030AST	30 m x 0.25 mm I.D., 0.12 μm
	Supelco DEX Column Screening Kit I – study the effect of CD size on selectivity Contains one each α-DEX 120, β-DEX 120, and γ-DEX 120
24340	30 m x 0.25 mm I.D., 0.25 μm
	Supelco DEX Column Screening Kit II – study the effect of phase on selectivity Contains one each β-DEX 120, β-DEX 225, γ-DEX 225, and β-DEX 325
24328-U	30 m x 0.25 mm I.D., 0.25 μm

* We offer a complete line of chiral and achiral derivatization reagents for GC.

Think Chiral - Think Sigma-Aldrich

Visit our chiral web portal sigma-aldrich.com/chiral to learn more about the wide range of products and services for chiral chemistry and separations from Supelco and other Sigma-Aldrich brands.

HPLC Column Screening & Method Optimization Guidelines

These screening protocols should provide a rapid determination of the most suitable column and mobile phase combination for an enantiomer separation. The optimization guidelines should fine-tune the separation.

Normal Phase (NP)	Analytes: All except extremely polar compounds		
CSP Type	Screening Mobile Phase	Optimization	Notes
Astec Cellulose DMP & Kromasil CelluCoat and AmyCoat <small>(and any cellulose or amylose phase)</small>	Isopropanol:Heptane (20:80)	<ul style="list-style-type: none"> Change the % polar modifier Try other alcohols (ethanol, IPA) Change both solvents 	<ul style="list-style-type: none"> Additives up to 0.1% v/v or w/v in the mobile phase are used to improve peak shape and selectivity Common modifiers include DEA, TEA, TFA, acetic acid, formic acid, and methanesulfonic acid
Astec CYCLOBOND <small>(DMP and DNP only)</small>	Ethanol:Heptane (30:70)	<ul style="list-style-type: none"> To sharpen peaks add 0.1% TFA (for acids) or 0.1% DEA or TEA (for bases) Try mixing acidic (e.g. 0.3% TFA) and basic additives (e.g. 0.2% TEA) 	
Astec P-CAP and P-CAP-DP	Ethanol:Heptane (20:80)		
Astec CHIROBIOTIC	Ethanol:Heptane (30:70)	<ul style="list-style-type: none"> Try ion pairing: For bases use methanesulfonic acid or ethanesulfonic acid as an additive 	
Cyclodextran (LARIHC)	Ethanol:Heptane (20:80)		

Reversed Phase (RP)	Analytes: All except extremely polar compounds		
CSP Type	Screening Mobile Phase	Optimization	Notes
Astec CHIROBIOTIC	CH ₃ OH or CH ₃ CN:20 mM ammonium acetate, pH 5 (30:70)	<ul style="list-style-type: none"> Change % and type of organic modifier Adjust pH, buffer type, ionic strength Acidic compounds, increase pH (6-7); neutral and basic compounds, decrease pH (3-4) 	<ul style="list-style-type: none"> LC/MS optimization: Use ammonium acetate or ammonium formate
Astec CYCLOBOND	CH ₃ CN:20 mM ammonium acetate, pH 5 (30:70) CH ₃ OH:20 mM ammonium acetate, pH 5 (20:80)	<ul style="list-style-type: none"> Change % and type of organic modifier Adjust pH, buffer type, ionic strength Acidic and neutral compounds, decrease pH (3-4) 	<ul style="list-style-type: none"> CH₃OH and CH₃CN can show large differences on CYCLOBOND in reversed-phase mode LC/MS optimization: Use ammonium acetate or ammonium formate
CHIRAL-AGP	CH ₃ CN:20 mM ammonium acetate, pH 4.5 (10:90)	<ul style="list-style-type: none"> Change % and type of organic modifier Adjust pH, buffer type, ionic strength 	<ul style="list-style-type: none"> Do not exceed 20% organic solvent on protein-based CSPs Try CHIRAL-CHB for bases, CHIRAL-HSA for acids

Polar Organic Mode (POM)	Analytes: Capable of hydrogen bonding		
CSP Type	Screening Mobile Phase	Optimization	Notes
Astec Cellulose DMP	Methanol containing 20 mM ammonium formate	<ul style="list-style-type: none"> Use methanol-ethanol blends Add 5-10% of other alcohols or acetonitrile 	<ul style="list-style-type: none"> Ammonium formate is good as an LC/MS additive
Astec CHIROBIOTIC	Methanol (neutral molecules)	<ul style="list-style-type: none"> Use other polar organic solvents or blends (e.g. combinations of CH₃CN, CH₃OH, ethanol, MTBE) 	<ul style="list-style-type: none"> Methanol should be the dominant solvent with CHIROBIOTIC in POM
Astec CYCLOBOND	CH ₃ CN:CH ₃ OH:acetic acid:TEA (95:5:0.1:0.1)	<ul style="list-style-type: none"> Test acid:base ratios from 1:4 to 4:1 to alter retention and selectivity. Typical acid and base concentrations are 0.01 to 1% 	<ul style="list-style-type: none"> Acetonitrile should be the dominant solvent with CYCLOBOND in POM LC/MS optimization: Replace TEA with ammonium hydroxide, lower concentration by 50-75%
Astec P-CAP/P-CAP-DP	CH ₃ CN:CH ₃ OH (70:30) (neutrals) 10 mM ammonium acetate in CH ₃ CN:CH ₃ OH (95:5) (polar cpds.)	<ul style="list-style-type: none"> Change the CH₃CN:CH₃OH ratio Increase ammonium acetate concentration 	
Cyclodextran (LARIHC)	CH ₃ CN:CH ₃ OH:acetic acid:TEA (60:40:0.3:0.2) CH ₃ CN:CH ₃ OH:TFA:TEA (90:10:0.3:0.2)	<ul style="list-style-type: none"> Change the CH₃CN:CH₃OH ratio Screen other additive combinations (e.g. TFA:TEA) Adjust acid:base ratio 	<ul style="list-style-type: none"> LARIHC CF6-P is highly selective for primary amines

Polar Ionic Mode (PIM)	Analytes: Ionizable compounds		
CSP Type	Screening Mobile Phase	Optimization	Notes
Astec CHIROBIOTIC	CH ₃ OH:acetic acid:TEA (100:0.1:0.1)	<ul style="list-style-type: none"> Test acid:base ratios from 1:4 to 4:1 to alter retention and selectivity. Typical acid and base concentrations are 0.01 to 1%. Acidic compounds, add base (use lower acid:base ratio); basic compounds, add acid (use e.g. ammonium acetate, ammonium formate, higher acid:base ratio) Change the type of acid or base Replace acid and base with a volatile salt, concentration 0.005 to 0.5% (can be tested using a concentration gradient). Try different salts. Acetonitrile can be added up to 50% 	<ul style="list-style-type: none"> Diethylamine (DEA) or NH₃OH can replace TEA, but selectivity will be different. LC/MS Optimization: Use volatile salts (e.g. ammonium acetate, ammonium formate, ammonium TFA)

SFC	Analytes: All except extremely polar compounds		
CSP Type	Screening Mobile Phase	Optimization	Notes
Astec Cellulose DMP	20% Methanol in CO ₂	<ul style="list-style-type: none"> Change methanol concentration Basic compounds, add diethylamine (DEA, 0.1%); acidic compounds, add trifluoroacetic acid (TFA, 0.2%) 	<ul style="list-style-type: none"> Run methanol scouting gradient 10 - 70% Neutral compounds do not need additives
Astec CHIROBIOTIC	30% Methanol in CO ₂ 30% CH ₃ OH:TFA:TEA (100:0.2:0.3) in CO ₂	<ul style="list-style-type: none"> Change methanol concentration Change TFA-TEA ratio for ionizable compounds 	<ul style="list-style-type: none"> Run methanol scouting gradient 10 - 70% Neutral compounds do not need additives
LARIHC	20% Methanol in CO ₂	<ul style="list-style-type: none"> Change methanol concentration For ionizable compounds use 0.3% TFA:0.2% TEA added to the methanol 	<ul style="list-style-type: none"> Run methanol scouting gradient 10 - 70% Neutral compounds do not need additives

General Method Development Notes

- Do not operate outside the phase's recommended range of solvents, temperature, pressure, etc.
- Allow 10 column volumes for equilibration in new mobile phase. CHIROBIOTIC columns can take longer (1-2 hours) than the typical 10 column volumes to equilibrate. In addition, when changing the mobile phase ratio, equilibration will need to be repeated.
- Move to next mobile phase system or column if there is no elution after 30 minutes, or if only a single, sharp peak elutes.
- Temperature: Increased temperature generally increases efficiency and improves peak shape. Decreased temperature generally increases chiral selectivity (enhances the weaker bonding forces). If operating from 50 - 70 °C, depending on how harsh the mobile phase, increase the temperature at 1 °C/min. maximum. Higher temperatures can reduce column lifetime, especially at pH extremes. Maintain temperature to within +/- 1 °C to maximize reproducibility.
- Flow rates: Chiral separations usually benefit from lower flow rates. Optimum flow rate is compound dependent and could be as low as 0.2 mL/min for a 4.6 mm ID Column. Test optimum for your separation.

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