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. 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.

	Chiral Phase					
Application/Field of Use	**Astec Cellulose DMP & Kromasil® ◆ Cellucoat™ DMPC-derivatized cellulose Column**	**Astec CHIROBIOTIC® Macrocyclic glycopeptides Column**	**Astec CYCLOBOND® Native & derivatized cyclodextrins Column**	**Astec CLC Copper ligand exchange Column**	**CHIRAL-AGP, HSA, CBH Immobilized proteins Column**	**Astec CHIRALDEX® & Supelco DEX™ Derivatized cyclodextrins for GC Column**
Routine Chiral Column Screening	●	●	●	●	●	●
Normal Phase HPLC	●	●	●	●	●	●
SFC	●	●	●	●	●	●
Reversed-Phase HPLC	●	●	●	●	●	●
Hydrophilic Interaction Liquid Chromatography (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/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

Type of CSP	Chiral Selectors (Phases)	Product Line
Polysaccharide	tris-(3,5-Dimethylphenyl) carbamoyl cellulose	Astec Cellulose DMP, Kromasil CelluCoat Columns
	tris-(3,5-Dimethylphenyl) carbamoyl amylose	Kromasil◆ AmyCoat™ Column
Macrocyclic glycopeptide	vancomycin, teicoplanin, teicoplanin aglycone, ristocetin A	Astec CHIROBIOTIC® Column
Cyclodextrin	ß- and γ-cyclodextrins, native and derivatized	Astec CYCLOBOND® Column
Protein	α_1-acid glycoprotein, cellobiohydrolase, human serum albumin	CHIRAL-AGP, CHIRAL-CBH, CHIRAL-HSA Columns
Chiral synthetic polymer	O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-L-tartar diamide	Kromasil◆ Chiral DMB Column
	O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide	Kromasil◆ Chiral TBB Column
Chiral ligand exchange	chiral bidentate ligand	Astec CLC-L, Astec CLC-D Columns

◆ Kromasil products -- analytical, prep, and bulk (up to 5 kg), are available exclusively through MilliporeSigma only in the U.S., Canada, and Puerto Rico.

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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.

HLPC Column Screening Kit*

Cat. No.	Description
10300AST	**Astec CHIROBIOTIC Column Screening Kit** *Contains one each Astec CHIROBIOTIC V2, T, R, and TAG* Columns 10 cm x 4.6 mm I.D., 5 µm
10305AST	25 cm x 4.6 mm I.D., 5 µm

GC Column Screening Kit*

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

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

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 Columns (and any cellulosic or amylosic phase)	Isopropanol:Heptane (20:80)	• Change the % polar modifier • Try other alcohols (ethanol, IPA) • Change both solvents (e.g. IPA for ethanol, test any organic solvent) • 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) • Try ion pairing: For bases use methanesulfonic acid or ethanesulfonic acid as an additive	• 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 Column (DMP and DNP only)	Ethanol:Heptane (30:70)		
Astec CHIROBIOTIC Column	Ethanol:Heptane (30:70)		

Reversed Phase (RP)	Analytes: All except extremely polar compounds		
CSP Type	**Screening Mobile Phase**	**Optimization**	**Notes**
Astec CHIROBIOTIC Column	Methanol or Acetonitrile:20 mM ammonium acetate, pH 5 (30:70)	• 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)	• LC-MS optimization: Use ammonium acetate or ammonium formate
Astec CYCLOBOND Column	Acetonitrile:20 mM ammonium acetate, pH 5 (30:70) Methanol:20 mM ammonium acetate, pH 5 (20:80)	• Change % and type of organic modifier • Adjust pH, buffer type, ionic strength • Acidic and neutral compounds, decrease pH (3-4)	• Methanol and Acetonitrile can show large differences on CYCLOBOND Column in reversed-phase mode • LC-MS optimization: Use ammonium acetate or ammonium formate
CHIRAL-AGP Column	Acetonitrile:20 mM ammonium acetate, pH 4.5 (10:90)	• Change % and type of organic modifier • Adjust pH, buffer type, ionic strength	• Do not exceed 20% organic solvent on protein-based CSPs • Try CHIRAL-CHB Column for bases, CHIRAL-HSA Column for acids

Polar Organic Mode (POM)	Analytes: Capable of hydrogen bonding		
CSP Type	**Screening Mobile Phase**	**Optimization**	**Notes**
Astec Cellulose DMP Column	Methanol containing 20 mM ammonium formate	• Use methanol-ethanol blends • Add 5-10% of other alcohols or acetonitrile	• Ammonium formate is good as an LC-MS additive
Astec CHIROBIOTIC Column	Methanol (neutral molecules)	• Use other polar organic solvents or blends (e.g. combinations of Acetonitrile, methanol, ethanol, MTBE)	• Methanol should be the dominant solvent with CHIROBIOTIC Column in POM
Astec CYCLOBOND Column	Acetonitrile:Methanol:acetic acid:TEA (95:5:0.1:0.1)	• 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%	• Acetonitrile should be the dominant solvent with CYCLOBOND Column in POM • LC-MS optimization: Replace TEA with ammonium hydroxide, lower concentration by 50-75%

Polar Ionic Mode (PIM)	Analytes: Ionizable compounds		
CSP Type	**Screening Mobile Phase**	**Optimization**	**Notes**
Astec CHIROBIOTIC Column	Methanol:acetic acid:TEA (100:0.1:0.1)	• 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%	• Diethylamine (DEA) or Ammonium Hydroxide 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 Column	20% Methanol in CO2	• Change methanol concentration • Basic compounds, add diethylamine (DEA, 0.1%); acidic compounds, add trifluoroacetic acid (TFA, 0.2%)	• Run methanol scouting gradient 10 - 70% • Neutral compounds do not need additives
Astec CHIROBIOTIC Column	30% Methanol in CO2 30% Methanol:TFA:TEA (100:0.2:0.3) in CO2	• Change methanol concentration • Change TFA-TEA ratio for ionizable compounds	• 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.

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