

CHIRAL HPLC COLUMNS

Columns for versatile
and robust Chiral HPLC
and LC-MS separations

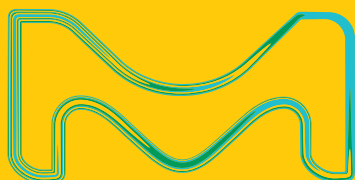


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Introduction: What is Chirality and Why is it Important?

Chirality belongs to the discipline of stereochemistry, which is the study of the three-dimensional structure of molecules. Chiral compounds are optically active, that means they rotate polarized light to the left or to the right depending on their configuration. The word comes from the Greek stem "chir-" meaning hand, for handedness. Chiral molecules are like left and right hands – they are mirror images. With no amount of rotation can you make the two images or molecules overlap. A chiral compound will rotate the plane of polarized light; the degree to which it does this is called the specific rotation or optical rotation.

Stereoisomers (spatial isomers) are isomers of the same substance that only differ in the spatial arrangement of their atoms. The maximum number of stereoisomers that can exist for a compound is 2^n , where n is the number of chiral centers. Chiral centers are atoms, usually carbon, bonded to four different groups. Stereoisomers that differ in the direction they rotate a plane of polarized light are called optically active, or chiral, and their isomers are called enantiomers. All enantiomers are stereoisomers but not all stereoisomers are enantiomers. Diastereomer (or diastereoisomer) are molecules that possesses more than one chiral center. Diastereomers may be enantiomers if they are mirror images. If not, they are just diastereomers which DO differ in chemical properties and can be separated by conventional means. Anomers are diastereomers that differ only in the configuration of the first carbon atom. Epimers are diastereomers that differ only in the configuration of the second C atom.

Besides the fact that one enantiomer is often safer and more efficacious than the other enantiomer, there are other arguments for having optically pure compounds. (1) Dosing is lower. If the product contains unwanted or inactive enantiomer, then they need to dose twice as much than they would if they had only the pure active enantiomer. (2) No interference of the desired activity by the unwanted enantiomer. In many cases, the unwanted enantiomer will have different biological activity and will interfere with the performance of the intended enantiomer. (3) Time savings in testing. If their product contains more than one enantiomer, they need to check the biological activity of each isomer plus the racemate to check for cooperative effects. This is three times the work than testing the pure enantiomer! These arguments are true for other industries besides pharmaceutical, for example agrochemicals. This fact has environmental implications as it can affect the total amount of chemical applied to the crop.

Astec® CHIROBIOTIC® HPLC Columns

Versatile Chiral HPLC and LC-MS Separations of Polar, Ionizable and Neutral Compounds

Astec® CHIROBIOTIC® CSPs (chiral stationary phases) interact with polar, ionizable and neutral analytes via multiple molecular interactions. This versatility means that the same Astec® CHIROBIOTIC® column can be successfully used in a variety of mobile phases, a significant benefit over CSPs that operate only in a single mode, normal or reversed-phase, for example, and must be dedicated to those mobile phase systems. However, the most interesting feature of Astec® CHIROBIOTIC® CSPs is the presence of ionic interactions, which allows them to be used in polar ionic and reversed-phase modes for sensitive LC-MS operation.

Key application areas

- Drug Discovery – High enantioselectivity, fast screening protocols, scalability to prep, reproducibility for reliable methods, polar and non-polar analytes
- Organic Synthesis – Compatible with all HPLC solvents to optimize sample solubility, fully scalable to prep
- Bioanalytical, Drug Metabolism – High throughput, MS-compatibility, aqueous samples, short run times, rugged columns
- Amino Acid and Peptide Analysis – Resolves underivatized natural and synthetic chiral amino acids and peptides, different selectivity and higher preparative capacity than C18 for achiral amino acids

What is the Astec® CHIROBIOTIC® family?

Developed originally by Advanced Separations Technologies (Astec®), the Astec® CHIROBIOTIC® family comprises highly enantioselective CSPs based on macrocyclic glycopeptides that have been bonded through multiple covalent linkages to high purity silica particles. Astec® CHIROBIOTIC® CSPs offer flexibility in choice of mobile phase conditions, both aqueous and non-aqueous, and are ideal for analytical

and preparative separations of neutral, polar and ionic compounds.

How do Astec® CHIROBIOTIC® CSPs separate enantiomers?

Astec® CHIROBIOTIC® CSPs offer six different types of molecular interactions: ionic, H-bond, pi-pi, dipole, hydrophobic, and steric. They also possess multiple inclusion sites that influence selectivity based on the molecular shape of the analyte. The optimization of enantiomer resolution is achieved by changing the mobile phase to leverage the types and relative strengths of the various interactions.

What makes Astec® CHIROBIOTIC® CSPs unique?

The bonded macrocyclic glycopeptide itself, in terms of its morphology, molecular composition, and multiple covalent linkages to the silica surface, is what makes Astec® CHIROBIOTIC® CSPs unique and gives them significant and valuable benefits over other CSPs. The truly differentiating feature of Astec® CHIROBIOTIC® CSPs is the presence of ionic interactions. These interactions are unique to Astec® CHIROBIOTIC® CSPs and are responsible in large part for their desirable retention characteristics toward polar and ionizable analytes in aqueous and non-aqueous solvents.

How do the Astec® CHIROBIOTIC® CSPs differ?

The various Astec® CHIROBIOTIC® phases share the benefits of robustness, flexibility in mobile phase options, ionic interactions, compatibility with polar compounds and LC-MS, and preparative scalability. However, Astec® CHIROBIOTIC® CSPs differ in selectivity, primarily because of their differing number and types of interaction sites, and the number, type and accessibility of ionic sites in the bonded macrocyclic glycopeptide.

The Astec® CHIROBIOTIC® CSP Family

Astec® CHIROBIOTIC® CSPs are based on 5 µm, high-purity, porous silica gel. These CSPs differ in the nature of the bonded macrocyclic glycopeptide and resulting enantioselectivity.

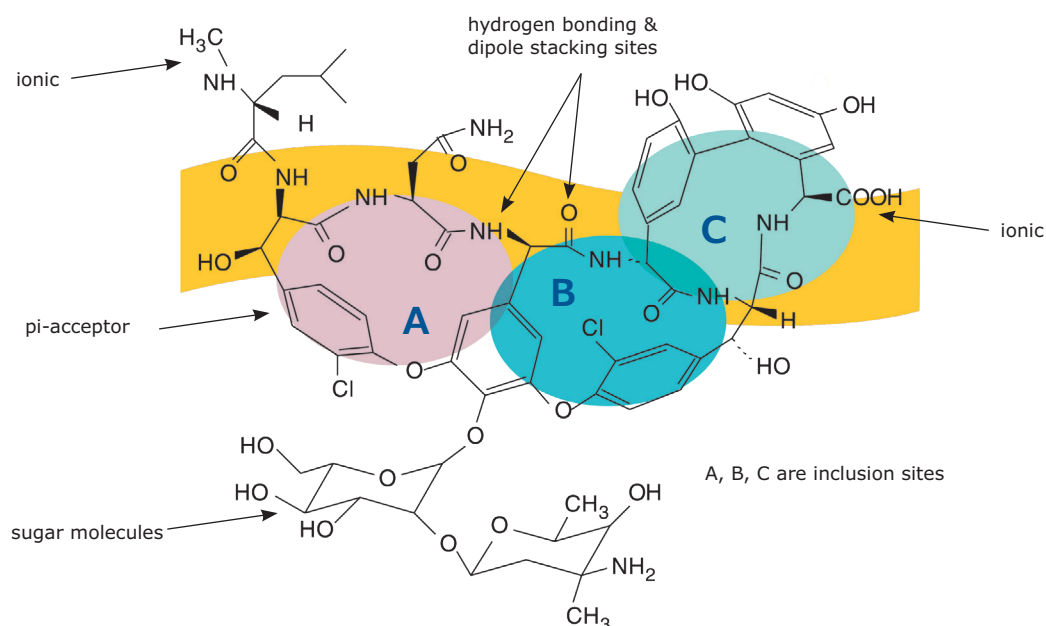
*Astec® CHIROBIOTIC® V and T differ from V2 and T2, respectively, in their bonding chemistry that gives them different selectivity and preparative capacity for certain classes of analytes.

- Astec® CHIROBIOTIC® V and V2* – Vancomycin
- Astec® CHIROBIOTIC® T and T2* – Teicoplanin
- Astec® CHIROBIOTIC® R – Ristocetin
- Astec® CHIROBIOTIC® TAG – Teicoplanin Aglycone

Key features of Astec® CHIROBIOTIC® CSPs:

- Aqueous and non-aqueous separations on the same column – Astec® CHIROBIOTIC® CSPs have H-bond, ionic, dispersive, π - π , dipole stacking, steric, and inclusion mechanisms, usually multiple types of interactions per analyte.
- Wide applicability – Applications cover a very broad range of compound classes, with the different Astec® CHIROBIOTIC® CSPs showing complementary selectivity.
- LC-MS compatibility – The wide choice of mobile phases makes Astec® CHIROBIOTIC® CSPs ideal for LC-MS, where analyte ionization and detection sensitivity are of critical concern.
- No solvent or additive memory effect – The same Astec® CHIROBIOTIC® column can be used alternately in polar, reversed-phase and normal phase solvents without damage, unlike cellulosic and amylosic phases that require dedicated operation.
- Robust columns with long lifetimes – Each macrocyclic glycopeptide molecule is linked to the silica surface via four or five covalent bonds for exceptional stability and long column life. The columns are designed to withstand high pressure and flow rates as well as rapid changes in mobile phase conditions.
- Solvent choices maximize sample solubility – Astec® CHIROBIOTIC® CSPs operate in highly-aqueous and non-aqueous polar mobile phases for polar compound solubility. The columns also operate in normal phase mobile phases to maximize solubility of non-polar compounds. Astec® CHIROBIOTIC® CSPs are compatible with all organic solvents.
- Excellent preparative scalability and capacity – From narrowbore to prep, separations on Astec® CHIROBIOTIC® columns are fully scalable, even with polar analytes. By relying on primarily aqueous eluents, the use and disposal of toxic organic solvents are eliminated. Additionally, preparative methods in the non-aqueous polar ionic mode are just as easy to process as normal phase solvents.
- Fast kinetics for speed and efficiency – The kinetics of the molecular interactions between the analyte and the Astec® CHIROBIOTIC® CSP are fast, providing efficient separations and relatively short retention times.
- Orthogonal selectivity to other CSPs – The six Astec® CHIROBIOTIC® columns CSPs are different from each other, and from other types of CSPs to offer choices in enantioselectivity, like reversal of elution order.

Proposed Structure of Vancomycin-based Astec® CHIROBIOTIC® V and V2



Incorporating Astec® CHIROBIOTIC® CSPs into Your Chiral Column Screening Protocol

It is recommended that you incorporate Astec® CHIROBIOTIC® columns into your routine screening protocol. Experience has shown that one or more of the Astec® CHIROBIOTIC® CSPs, particularly V2, T and TAG, will perform the majority of chiral separations. Even if other CSPs give adequate resolution, an Astec® CHIROBIOTIC® CSP may allow use of mobile phases that are better suited to your sample and detection method, or the Astec® CHIROBIOTIC® method may be faster, more efficient, or more robust. An Astec® CHIROBIOTIC® method may also have advantages from a preparative standpoint in terms of solvent selection and sample capacity.

For developing a new chiral HPLC method, we have created and use routinely in our laboratories a simple and rapid chiral column screening protocol.

The Astec® CHIROBIOTIC® Screening Protocol

columns: Astec® CHIROBIOTIC® V2, T, R, and TAG

procedure: Method development follows a simple strategy that tests polar ionic, polar organic, reversed-phase, and normal phase modes.

Separation Mode	Description	Types of Compound	Screening Mobile Phase	Parameters to Optimize
Polar Ionic	Polar organic solvents Methanol or Acetonitrile containing small amounts of acid and base or salt	Acids, Bases, Zwitterions	(100:0.1:0.1, v:v:v) Methanol:Acetic Acid: Triethylamine	Change acid-base ratio, change the type of acid or base, add a volatile salt (test different ammonium salts)
Reversed-Phase (RP)	Typical RP eluents, water or buffers with Methanol or Acetonitrile	Acids, Bases, Zwitterions, Neutrals	(30:70) Acetonitrile:20 mM Ammonium Acetate, pH 4.0	Change the % and type of organic modifier, adjust pH, buffer type, and ionic strength
Polar Organic	Polar organic solvents without ionic additives	Neutrals	100% Ethanol	Use other polar organic solvents or blends
Normal Phase	Non-polar organic solvents with polar solvent modifiers	Neutrals	(30:70) Ethanol:Heptane	Increase % of polar modifier, change both solvents

Method Optimization: Acid-Base Ratio, Temperature, and Flow Rate in Polar Ionic Mode

Using Astec® CHIROBIOTIC® CSPs in the polar ionic mode has the highest probability of success. Optimizing resolution usually involves changing the contribution to retention of ionic interactions between the analytes and functional groups in the macrocyclic glycopeptide structure by:

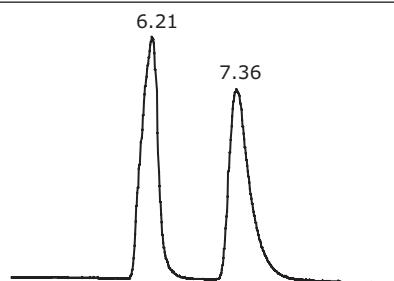
- Changing the ratio of acid to base
- Adding a soluble, volatile salt (instead of the acid and base) directly to the methanol

The acid, base, or salt that is ultimately selected is based on its compatibility, with the detection method (e.g. LC-MS), sample solubility, and whether the separation will be scaled up to preparative.

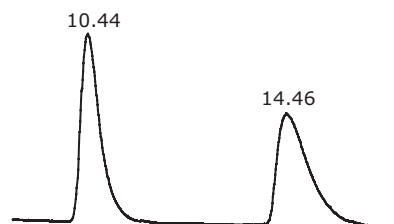
Demonstration of Polar Ionic Mode Mechanism: Effect of Acid:Base Ratio

Column	CHIROBIOTIC® V
Mobile Phase	Methanol/Acetic Acid/Triethylamine

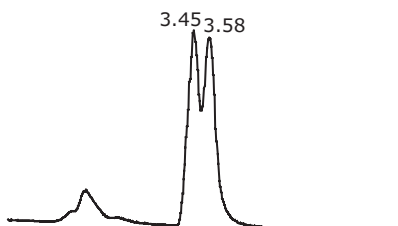
100/0.1/0.1
Acid:base ratio=1:1



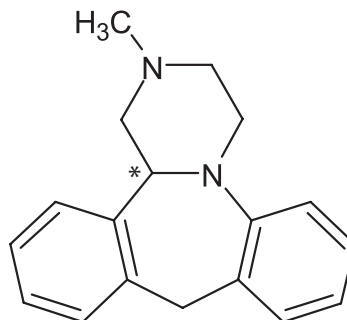
100/0.15/0.05
Acid:base ratio=3:1



100/0.05/0.15
Acid:base ratio=1:3



Mianserin



High acid: Nitrogen on mianserin is positively charged, while -COOH group on vancomycin is negatively charged: strong ionic interaction

High base: Nitrogen on mianserin group is free amine, but -COOH group on vancomycin is fully charged: weak ionic interaction

Astec® CHIROBIOTIC® columns: Ideally Suited for

LC-MS of Polar, Ionizable and Neutral Compounds

Each of the various ionization sources has an optimal set of mobile phase conditions. Outside this set, ionization may be suppressed with resulting loss in sensitivity. Astec® CHIROBIOTIC® phases are uniquely able to operate across all mobile phase systems. CSPs that are limited to normal phase operation, like many cellulosic and amylosic CSPs, reduce the options in detection methods.

ESI – Operate Astec® CHIROBIOTIC® CSPs in reversed-phase and unique polar ionic modes.

APCI – Operate Astec® CHIROBIOTIC® CSPs in polar ionic mode.

APPI – Operate Astec® CHIROBIOTIC® CSPs in normal phase mode.

Typical polar ionic mobile phases are methanol with low concentrations (0.1 – 0.001%) of volatile salts, like ammonium acetate or ammonium formate. The applications below show examples of Astec® CHIROBIOTIC® CSPs for LC-MS in reversed-phase and polar ionic mode mobile phases, respectively.

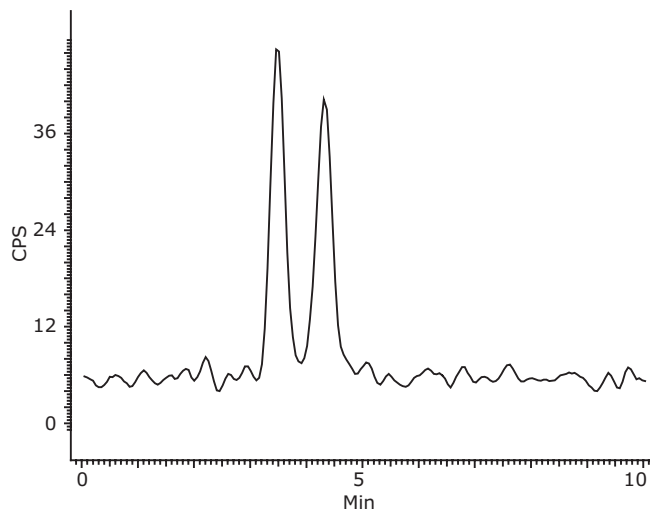
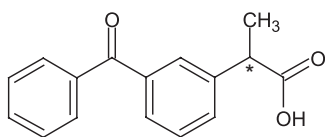
In addition to mobile phase compatibility, the allowable high flow rates and short columns make them ideally suited to fast MS applications.

Astec® CHIROBIOTIC® columns can be used in conjunction with HybridSPE®-PPT plates to enhance sensitivity by completely removing endogenous proteins and phospholipids. This approach was used to resolve the enantiomers of clenbuterol on an Astec® CHIROBIOTIC® T column.

ESI-MS of Ketoprofen on Astec® CHIROBIOTIC® R in Reversed-phase Mode

column:	Astec® CHIROBIOTIC® R, 15 cm x 2.1 mm I.D., 5 µm (13019AST)
mobile phase:	(30:70) Methanol:20 mM ammonium acetate, pH 5.6
flow rate:	0.2 mL/min
det.:	ESI(-)
temp.:	35 °C
analyte:	Ketoprofen

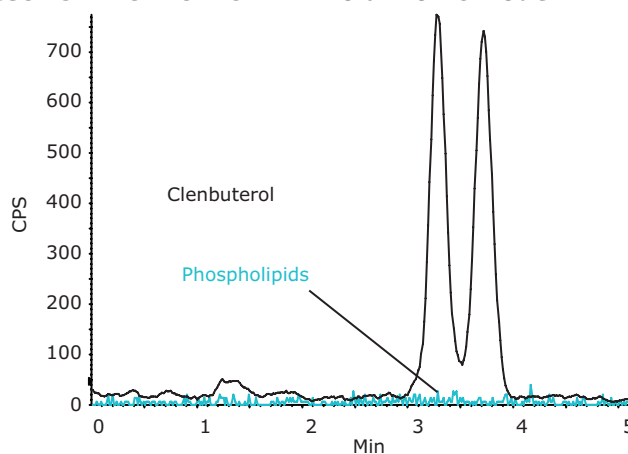
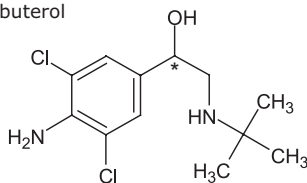
Ketoprofen



ESI-MS of Clenbuterol Extracted from Plasma on Astec® CHIROBIOTIC® T in Polar Ionic Mode

column:	Astec® CHIROBIOTIC® T, 10 cm x 2.1 mm I.D., 5 µm (12018AST)
mobile phase:	10 mM ammonium formate in Methanol
flow rate:	0.3 mL/min
det.:	ESI(+)
temp.:	30 °C
analyte:	Clenbuterol in rat plasma (10 ng/mL)

Clenbuterol



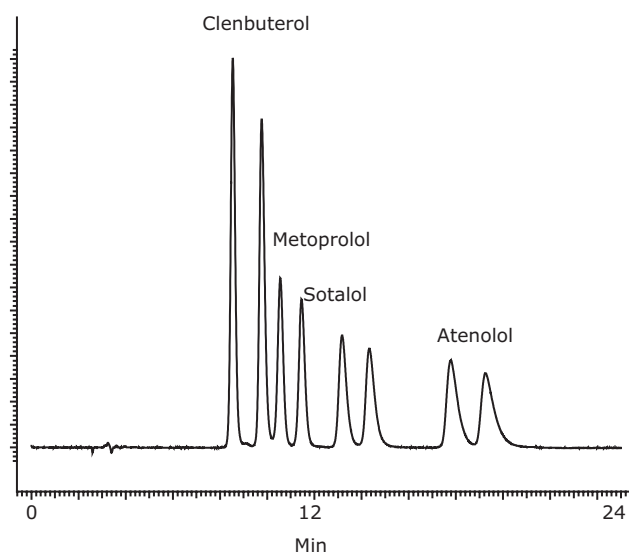
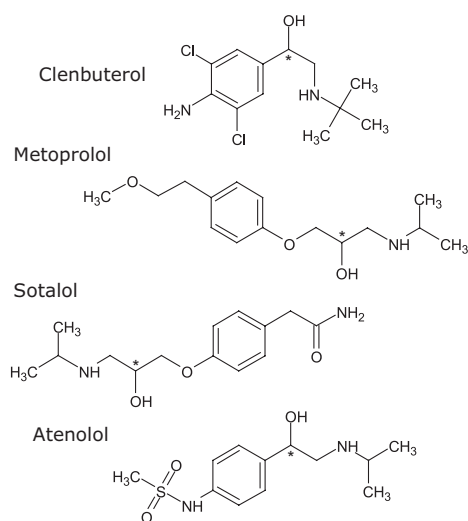
Unique Polar Ionic Mode

A valuable feature of Astec® CHIROBIOTIC® CSPs, the novel and versatile polar ionic mode, is popular because its mobile phases are polar, organic solvents, containing volatile additives, that are ideally suited for preparative and LC-MS applications. An example is

shown below. Additionally, compared to normal phase separations, the polar ionic mode has speed, efficiency, and sensitivity advantages, all valuable assets for LC-MS.

Beta-Receptors on Astec® CHIROBIOTIC® T in Polar Ionic Mode

column:	Astec® CHIROBIOTIC® T, 25 cm x 4.6 mm I.D., 5 µm (12024AST)
mobile phase:	15 mM ammonium formate in Methanol
flow rate:	1 mL/min
det.:	UV at 220 nm
temp.:	25 °C



Multi-modal Interactions Permit Use in Aqueous and Non-aqueous Solvents

All Astec® CHIROBIOTIC® CSPs possess multiple interaction sites on the same column. Changing the mobile phase affects the relative strength of specific types of interactions. The power and flexibility of multi-modal Astec® CHIROBIOTIC® CSPs are demonstrated in the following applications. The vancomycin-based Astec® CHIROBIOTIC® CSPs were used successfully in four different modes.

Polar Ionic Mode

A valuable feature of Astec® CHIROBIOTIC® columns, the novel and versatile polar ionic mode mobile phase system, is desirable because of its high volatility and beneficial ionization effect for LC-MS.

Reversed-phase Mode

Also highly suitable for LC-MS and polar analytes, reversed-phase (RP) is a mode familiar to all chromatographers. Astec® CHIROBIOTIC® CSPs have RP character and can be used in a wide range of buffers and solvent.

Polar Organic Mode

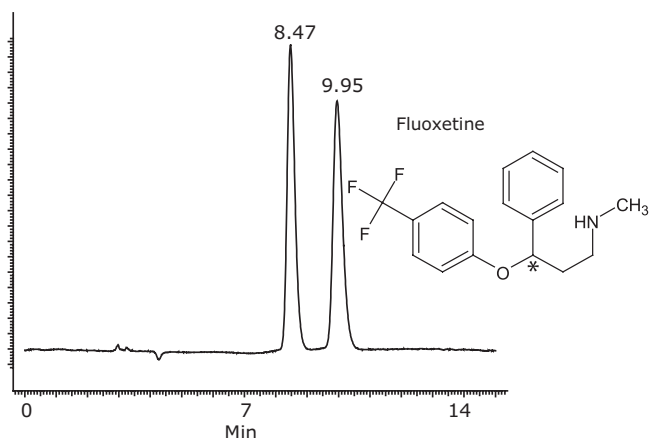
Enantiomers of polar neutral analytes have been successfully separated on Astec® CHIROBIOTIC® columns in the polar organic mode where the mobile phase is typically a polar organic solvent or solvent blend. Reaction mixtures, even in pyridine, can be run on Astec® CHIROBIOTIC® columns in this mode.

Normal Phase Mode

Normal phase chiral separations are desirable to maintain solubility of hydrophobic compounds and when analyzing reaction mixtures in non-polar organic solvents. Astec® CHIROBIOTIC® CSPs have the flexibility to operate in normal phase mode. The same column can be used with normal phase and polar/aqueous solvents and additives without memory effects.

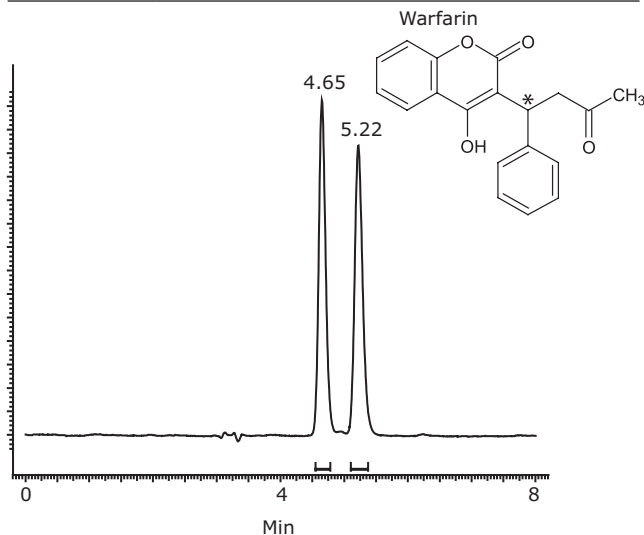
Polar Ionic Mode

column:	Astec® CHIROBIOTIC® V2, 25 cm x 4.6 mm I.D., 5 µm (15024AST)
mobile phase:	15 mM ammonium formate in Methanol
flow rate:	1 mL/min
det.:	UV at 230 nm
temp.:	25 °C
analyte:	Fluoxetine



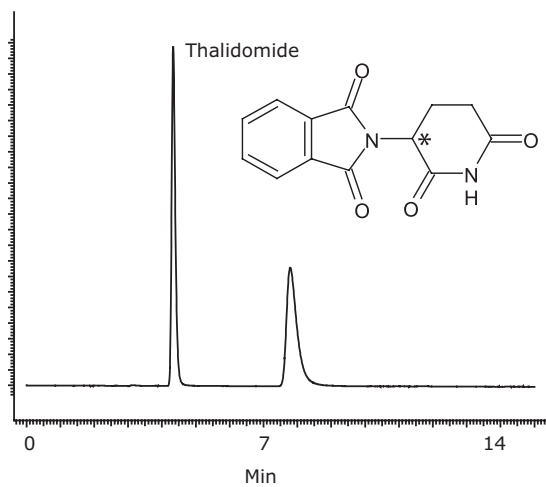
Reversed-phase Mode

column:	Astec® CHIROBIOTIC® V, 25 cm x 4.6 mm I.D., 5 µm (11024AST)
mobile phase:	(30:70) Acetonitrile:5 mM ammonium acetate, pH 4.1
flow rate:	1 mL/min
det.:	UV at 254 nm
temp.:	25 °C
analyte:	Warfarin



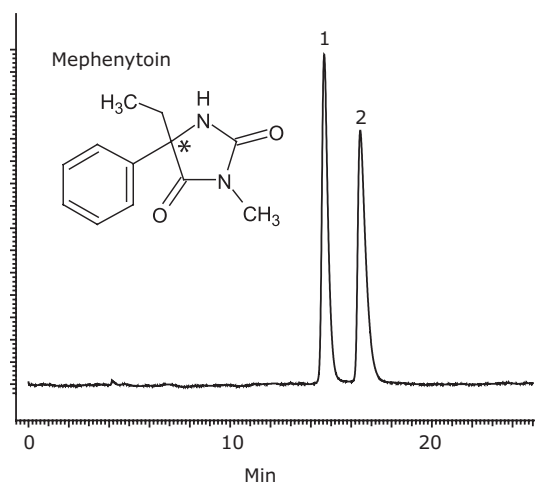
Polar Organic Mode

column:	Astec® CHIROBIOTIC® V2, 25 cm x 4.6 mm I.D., 5 μm (15024AST)
mobile phase:	Methanol
flow rate:	1 mL/min
det.:	UV at 230 nm
temp.:	25 °C
analyte:	Thalidomide



Normal Phase Mode

column:	Astec® CHIROBIOTIC® V, 25 cm x 4.6 mm I.D., 5 μm (11024AST)
mobile phase:	(95:5) hexane:ethanol
flow rate:	1 mL/min
det.:	UV at 205 nm
temp.:	25 °C
analyte:	Mephenytoin



Preparative Applications Using Astec® CHIROBIOTIC® CSPs

- Scalability across all Astec® CHIROBIOTIC® CSPs
- Low retention times give high throughput

Astec® CHIROBIOTIC® columns can be used in all preparative HPLC techniques, including elution and recycle chromatography, mass-directed prep, SFC, and simulated moving bed (SMB). Multiple covalent bonds attach the Astec® CHIROBIOTIC® macrocyclic glycopeptides to the silica surface, meaning no CSP ligand will contaminate the product. Preparative separations on Astec® CHIROBIOTIC® columns often have speed and efficiency benefits over other CSPs. In terms of loading capacity, a 25 cm x 21.2 mm I.D. column has medium to high loadings, from a few mg to over 300 mg per injection.

A significant advantage of Astec® CHIROBIOTIC® columns for preparative applications is the fact that the mobile phase can be chosen to optimize sample solubility – a critical preparative consideration. The examples here show preparative Astec® CHIROBIOTIC® separations in three different mobile phase systems.

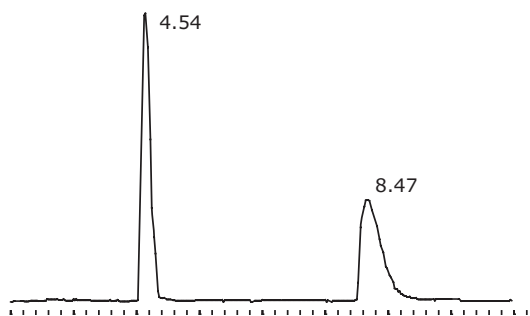
Preparative Separation on Astec® CHIROBIOTIC® TAG in Polar Ionic Mode

column:	Astec® CHIROBIOTIC® TAG, 25 cm x 21.2 mm I.D., 5 µm (14044AST)
mobile phase:	0.1% ammonium acetate in Methanol
flow rate:	35 mL/min
det.:	UV at 300 nm
throughput:	20 mg/g CSP/hr
load:	200 mg in 6 mL
analyte:	N-Acetyl Tryptophan

Sample Solubility Considerations in Preparative

Analytical Scale

column:	Astec® CHIROBIOTIC® V, 25 cm x 4.6 mm I.D., 5 µm (11024AST)
mobile phase:	Methanol
det.:	UV at 293 nm
flow rate:	1 mL/min
analyte:	Thalidomide

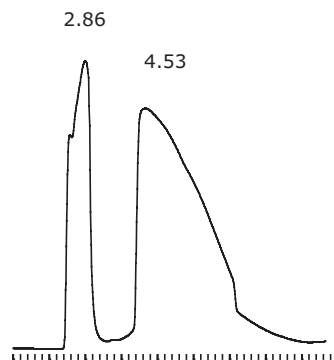


Preparative Reversed-phase and Polar Ionic Modes

Preparative separations in reversed-phase and polar ionic mode solvents have benefits over normal phase preparative separations in terms of solvent safety and waste disposal costs. The application below shows the use of Astec® CHIROBIOTIC® TAG columns in a preparative separation in polar ionic mode.

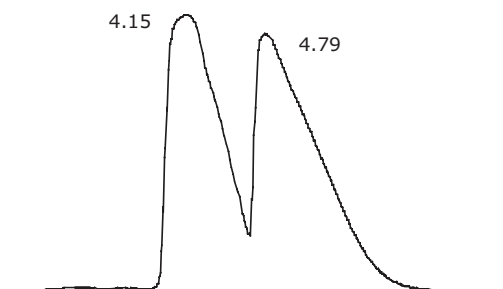
Preparative Polar Organic Mode

The application here shows the analytical and preparative separations of thalidomide enantiomers on Astec® CHIROBIOTIC® T columns. The analytical scale gave an α value of 3.35 in 100% methanol and a retention time under 10 minutes. However, since thalidomide is fairly insoluble in pure methanol, it was possible to add 20% dioxane to the mobile phase to increase solubility 3.5-fold while still achieving the necessary separation.



Prep Scale

column:	Astec® CHIROBIOTIC® V, 25 cm x 21.2 mm I.D., 5 µm (11044AST)
mobile phase:	(80:20) Methanol:dioxane
det.:	UV at 313 nm
flow rate:	20 mL/min
load:	70 mg in 12 mL
analyte:	Thalidomide



Astec® CHIROBIOTIC® V and V2

Bonded Macrocylic Glycopeptide:	Vancomycin
Chiral Centers:	18
Sugar Moieties:	2
Inclusion Cavities:	3

Separates a wide variety of secondary and tertiary amines in the polar ionic mode. Have many of the separation characteristics of protein-based stationary phases but with exceptional stability and much higher sample capacity. Astec® CHIROBIOTIC® V2 and V columns differ in the chemistry used to bond the glycopeptide to the silica, which gives them differences in selectivity.

Astec® CHIROBIOTIC® T and T2

Bonded Macrocylic Glycopeptide:	Teicoplanin
Chiral Centers:	23
Sugar Moieties:	3
Inclusion Cavities:	4

These CSPs have resolved all of the known beta-blockers and dihydrocoumarins and many other compound classes. Generally reproduces chiral crown ether or ligand-exchange for amino acid separations. Astec® CHIROBIOTIC® T2 and T columns differ in the chemistry used to bond the glycopeptide to the silica, which gives them differences in selectivity.

Astec® CHIROBIOTIC® TAG

Bonded Macrocylic Glycopeptide:	Teicoplanin Aglycone
Chiral Centers:	8
Sugar Moieties:	0
Inclusion Cavities:	4

The removal of the three sugar moieties enhances resolution of many of the amino acids (alpha, beta, gamma, and cyclic). Astec® CHIROBIOTIC® TAG columns has shown remarkable selectivity for sulfur-containing molecules, such as sulfoxides and the amino acids methionine, histidine, and cysteine. Neutral molecules, like oxazolidinones, hydantoins, and diazepines, have shown enhanced resolution and, more remarkably, in single-solvent systems like methanol, ethanol, and acetonitrile. Some acidic molecules have also shown increased selectivity.

Astec® CHIROBIOTIC® R

Bonded Macrocylic Glycopeptide:	Ristocetin A
Chiral Centers:	38
Sugar Moieties:	6
Inclusion Cavities:	4

The presence of amines in the ristocetin structure makes it a good choice when screening acidic compounds.

Astec® CHIROBIOTIC® Product Listing

Method Development Kits

ID (mm)	Length (cm)	Cat. No.	
4.6	10	10300AST	- One each of Astec® CHIROBIOTIC® V2, T, TAG and R
4.6	25	10305AST	- One each of Astec® CHIROBIOTIC® V2, T, TAG and R

(5 µm)

Length (mm)	I.D. (mm)	V	V2	T	T2	TAG	R
100 x	2.1	11018AST	15018AST	12018AST	16018AST	14018AST	
150 x	2.1	11019AST	15019AST	12019AST	16019AST	14019AST	13019AST
250 x	2.1	11020AST	15020AST	12020AST		14020AST	13020AST
100 x	3.0			12010AST			
50 x	4.6			12021AST			
100 x	4.6	11022AST	15022AST	12022AST		14022AST	13022AST
150 x	4.6	11023AST	15023AST	12023AST	16023AST	14023AST	13023AST
250 x	4.6	11024AST	15024AST	12024AST	16024AST	14024AST	13024AST
250 x	10	11034AST		12034AST		14034AST	
250 x	21.2	11044AST	15044AST			14044AST	
Guard 20	x 1.0	11101AST	15101AST	12101AST			13101AST
Guard 20	x 4.0	11100AST	15100AST	12100AST		14100AST	
Guard Holder	x			21150AST			

For more information and to review our complete offering of Astec® CHIROBIOTIC® columns, please visit SigmaAldrich.com

*Other column dimensions, including guard columns and preparative dimensions, are found on our website or by inquiring with technical service.

Astec® CYCLOBOND® HPLC Columns

The versatile and unique Astec® CYCLOBOND® CSPs (chiral stationary phases) are a family of derivatized and underivatized β - and γ -cyclodextrins bonded to high-purity silica gel. Patented by Professor Daniel W. Armstrong and introduced to the market in 1984, they have found widespread use for isomer separations by HPLC, both chiral and achiral. Astec® CYCLOBOND® is complementary to other CSPs, including the polysaccharide-based CSPs and macrocyclic glycopeptide-based Astec® CHIROBIOTIC® CSPs.

Key Features and Application Areas

- Ideal for chiral analysis in the pharmaceutical industry, and for small analytes in chemical and environmental areas
- Routine chiral column method development screening protocols
- All chromatography modes: Reversed-phase, polar organic, normal phase, and SFC
- Complementary selectivity to other types of CSPs
- Highly compatible with LC-MS
- Scalable from analytical to preparative
- Covalently bonded for long column lifetime

The Astec® CYCLOBOND® CSP Family

Name	Cyclodextrin	Derivative (2- and 3-position hydroxyls)
Astec® CYCLOBOND® I 2000	Beta (β)	None (native)
Astec® CYCLOBOND® I 2000 AC	Beta (β)	Acetyl
Astec® CYCLOBOND® I 2000 DMP	Beta (β)	3,5-Dimethylphenylcarbamate
Astec® CYCLOBOND® I 2000 SP	Beta (β)	S-Hydroxypropyl ether
Astec® CYCLOBOND® I 2000 RSP	Beta (β)	R,S-Hydroxypropyl ether
Astec® CYCLOBOND® I 2000 HP-RSP	Beta (β)	R,S-Hydroxypropyl ether
Astec® CYCLOBOND® II	Gamma (γ)	None (native)

In addition to the Astec® CYCLOBOND® columns family of cyclodextrin-based CSPs, we also offer a high-throughput option, Chiradex®. Please visit SigmaAldrich.com for more information.

What Makes Astec® CYCLOBOND® CSPs Unique?

CYCLOBOND® CSPs offer unique chiral selectivity by way of multiple chiral mechanisms provided by the cyclodextrin cavity and the functional groups of the various derivatives. CYCLOBOND® CSPs feature chemical stability for long lifetime, wide mobile phase choices, and high efficiency.

Astec® CYCLOBOND® I 2000 Series

- Native β -cyclodextrin and five β -cyclodextrin derivatives bonded to high-purity silica gel
- Excellent chiral selectors for substituted phenyl, naphthyl, and biphenyl compounds

Astec® CYCLOBOND® II Series

- Native γ -cyclodextrin bonded to high-purity silica gel
- Excellent chiral selectors for multi-ring structures, such as those based on anthracene, chrysene, or pyrene

Combining the Power of Cyclodextrin Architecture and Selective Surface Chemistry

What are Cyclodextrins?

Cyclodextrins are produced by partial degradation of starch, followed by the enzymatic coupling of glucose units into crystalline, homogeneous toroidal structures of different molecular size. The D(+)-glucose residues are bonded to each other through α -(1,4)glycosidic linkages. The chair configuration of glucose residues makes the toroid "bucket" narrower at one end (see below). Three highly-characterized cyclodextrins are alpha (α), beta (β), and gamma (γ) cyclodextrin, which contain six, seven, and eight glucose units, respectively (see below table). Because each glucose residue has five chiral centers, cyclodextrins are themselves chiral structures. For example, β -cyclodextrin has 35 chiral centers.

How Do Cyclodextrin-based CSPs Separate Enantiomers?

Both the architecture and chemistry of cyclodextrins contribute to enantiomer separations. The toroidal cyclodextrin structure has a hydrophilic exterior surface resulting from the 2-, 3-, and 6-position hydroxyl (OH) groups. The interior cyclodextrin cavity is composed of the glucose oxygens and methylene hydrogens, which gives it a non-polar (hydrophobic) character. Chemical interactions that lead to chiral separations occur on both the exterior and interior surfaces of the cyclodextrin toroid. The most important consideration

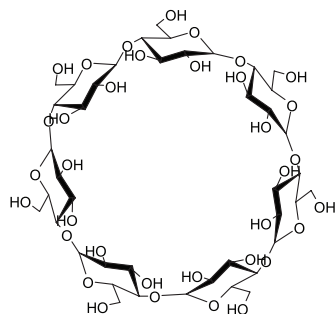
for retention and chiral recognition is proper fit of the analyte into the cyclodextrin cavity. This fit is a function of both molecular size and shape of the analyte relative to the cyclodextrin cavity. Thus, there are two basic mechanisms at play in chiral separations on cyclodextrins: those that occur on the inside cavity surface (inclusion complexing) and those that occur on the outside surface (surface interactions) of the cyclodextrin toroid.

Mechanism 1: Inclusion Complexing

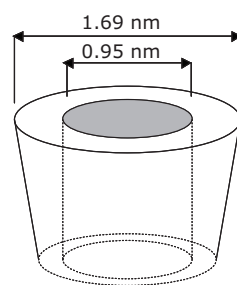
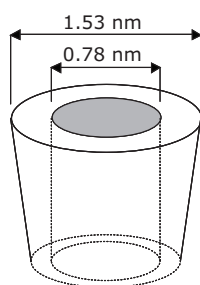
The basis for many separations on cyclodextrin-based CSPs in the reversed-phase mode (mobile phases containing water with methanol or acetonitrile) is a phenomenon called inclusion complexing. If the analyte can fit into the cyclodextrin cavity and mobile phase conditions are favorable, the inclusion complexing mechanism can occur. It is because of inclusion complexing that reversed-phase is a very successful mode on CYCLOBOND® CSPs. Three points of interaction are required for a chiral discrimination, and the inclusion complexing provides one of the three interactions.

The inclusion complexing mechanism is attributed to the attraction of the apolar molecule or segment of the molecule to the apolar cyclodextrin cavity, which is sensitive to structural differences. When the analyte possesses an aromatic

Proposed Structure of Cyclodextrin Molecules



Cycloheptylamylose (β -Cyclodextrin)



Cyclooctylamylose (γ -cyclodextrin)

Properties of Cyclodextrins

Cyclodextrin	Chemical Name	Glucose Units	Stereogenic Centers	Cavity Size (nm)
Alpha (α)*	Cyclohexylamylose	6	30	0.57
Beta (β)	Cycloheptylamylose	7	35	0.78
Gamma (γ)	Cyclooctylamylose	8	40	0.95

* α -CDs are currently not available as CSPs in the Astec® line

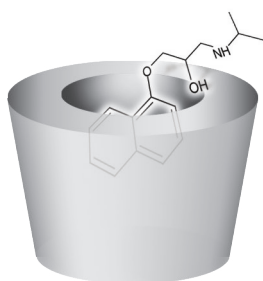
group, the orientation in the cavity is selective due to the sharing of electrons between the aromatic methylene groups and the glucoside oxygens on the internal surface of the cyclodextrin toroid. The mechanism is completed by interaction of solute functional groups with the 2- and 3- position secondary hydroxyl groups of the cyclodextrin ring. A schematic of the inclusion mechanism is shown in the figure. Linear or acyclic hydrocarbons occupy more random positions in the cavity. If a chiral separation is attempted in reversed-phase mode, it is therefore essential that the analyte have at least one aromatic ring or ring structure. The inclusion complexing mechanism also provides good separations of positional isomers. Inclusion complexing does not occur to the same extent in polar organic or normal phases modes because the non-polar attractions between analyte and the CD cavity are not favored.

Mechanism 2: Surface Interactions

In surface interactions, the chiral molecule lies across the external surface of the cyclodextrin toroid and interacts with the upper rim of the ring. Surface interactions dominate in polar organic (methanol or acetonitrile containing additives) and normal phase modes because in these modes analytes do not interact with the cyclodextrin cavity. This mechanism is for two reasons: First, when acetonitrile is present, it fully inserts into the cavity and blocks analytes from entering it. Second, when the mobile phase is totally non-aqueous, the non-polar interactions between analytes and the interior of the cyclodextrin cavity cannot occur. The surface interaction mechanism is depicted in the figure below.

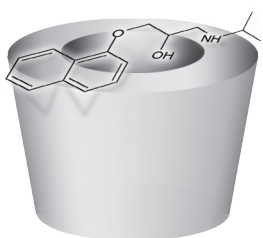
Inclusion Complexing Schematic

Representation of the inclusion complexing mechanism of an analyte into the cyclodextrin cavity. Subsequent interactions occur between the analyte and groups on the cyclodextrin surface. The analyte molecular size, shape and types of functional groups on it and the cyclodextrin contribute the enantioselectivity. Inclusion complexing occurs in reversed-phase mode.



Surface Interaction Schematic

Representation of the surface interaction mechanism of an analyte with the cyclodextrin. These interactions dominate in polar organic and normal phase modes.



Functional Group Interaction

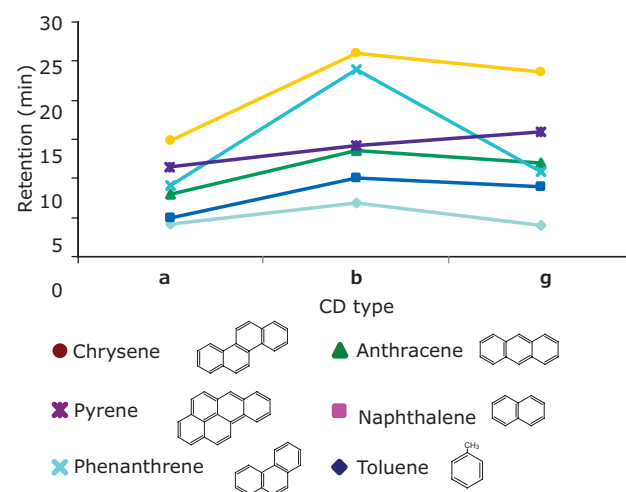
Certain analyte functional groups have a strong affinity for the cyclodextrin cavity. Other polar groups strongly hydrogen bond to the high-density hydroxyl surface of the native cyclodextrin. Derivatization of the cyclodextrin molecule at the 2- and 3-position hydroxyl groups affects selectivity and can be leveraged to alter the extent to which inclusion complexing occurs. For example, derivatized cyclodextrins, such as CYCLOBOND® I 2000 DMP (3,5-dimethylphenyl carbamate), provide additional interactions (π - π) as well as H-bonding. Halogens form strong inclusion complexes with these CSPs.

The affinity for the cyclodextrin cavity is influenced by functional groups, such as halogens, nitrates, sulfates, phosphates, and phenols on the analyte's aromatic rings. When these groups are present, inclusion complexing in reversed-phase mode is preferred. Protic substituents on the analyte, including carboxyls, carbonyls, amides, hydroxyls, and amines, generally provide surface interactions. Hydrogen bonding and dipole-dipole interactions also contribute to chiral selectivity.

Shape Selectivity

The graph below shows that for the cyclodextrin inclusion mechanism to occur, the molecular weight of a polyaromatic ring structure is not as critical as its footprint. The enantiomers of an analyte like norgestrel (a four-ring steroid structure) are better separated on the γ -cyclodextrin (CYCLOBOND® II series). The β -cyclodextrin (CYCLOBOND® I 2000 series) is a better option for enantiomers of naphthalene-like structures or singly-substituted aromatic ring structures.

Molecular Size Selectivity on Cyclodextrin CSPs (same chemistry, different cavity size)



What Types of Enantiomers are Separated on Astec® CYCLOBOND® CSPs?

In general, substituted phenyl, naphthyl, and biphenyl rings can be separated on β -cyclodextrin-based CYCLOBOND® I 2000 columns and its derivatives. Molecules with heterocyclic rings also often separate on these phases. Analytes with three to five rings, including steroids, are best separated on γ -cyclodextrin-based CYCLOBOND® II columns. Enantiomers with halogens, nitrates, sulfates, phosphates, and hydroxyls on the analyte's aromatic rings generally separate well on CYCLOBOND® CSPs. Also successfully resolved on CYCLOBOND® columns are compounds with hydrogen-bonding functional groups off a ring, cis/trans and positional isomers (see application below), closely-related achiral molecules, and derivatized chiral amino acids.

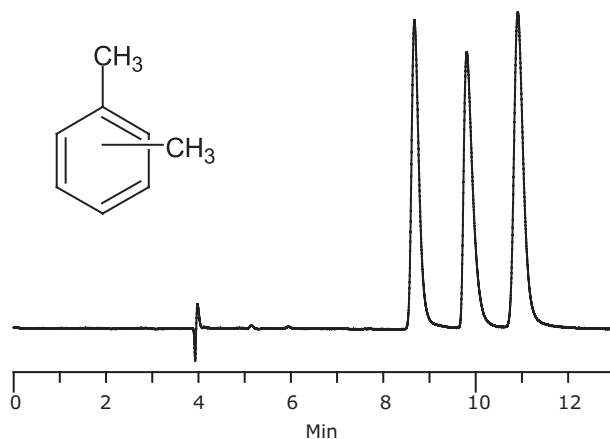
Mobile Phases for Astec® CYCLOBOND® CSPs

One of the important aspects of the CYCLOBOND® family is that it operates in all mobile phase systems, permitting choice based on analyte solubility, detection method, or operator preference. It is interesting to note that temperature has dramatic effects on chiral selectivity on cyclodextrin phases.

- Reversed-phase: Water or buffers containing methanol or acetonitrile. Generally favors inclusion complexing.
- Normal phase & SFC: Hexane or Carbon dioxide containing typical co-solvents (methanol, ethanol, isopropanol) and additives (Trifluoroacetic acid, triethylamine, diethylamine, etc.). Favors hydrogen bonding or π - π interaction on derivatized cyclodextrins (CYCLOBOND® DMP columns only).
- Polar organic: Acetonitrile and/or methanol containing additives (acetic acid and triethylamine) that control hydrogen bonding or peak tailing. This mode enhances interactions with secondary hydroxyl groups across the cyclodextrin ring opening, as well as some functional groups found on derivatized cyclodextrins.

Positional Isomers (Xylenes) on Astec® CYCLOBOND® I 2000

column:	CYCLOBOND® I 2000, 25 cm x 4.6 mm I.D., 5 μ m (20024AST)
mobile phase A:	acetonitrile
mobile phase B:	water
mobile phase ratio:	15:85 (A:B)
flow rate:	0.8 mL/min
temp.:	45 °C
det.:	UV, 230 nm
injection:	3 μ L
sample:	each compound, 0.1 mg/mL in acetonitrile:water (50:50)
elution order:	m-, o-, p-xylene



Incorporating Astec® CYCLOBOND® into Your Chiral Column Screening Protocol

We recommend incorporating CYCLOBOND® CSPs into your chiral column screening protocol. The CSP's unique selectivity makes them complementary to other CSPs and may provide the extra resolution needed to separate the target enantiomers. The table below outlines our recommended method development screening protocol for CYCLOBOND® columns in the different mobile phase systems.

Astec® CYCLOBOND® Screening Protocol

Mobile Phase System	Starting Composition	Optimization
Reversed-phase (RP)	Methanol or Acetonitrile / 20 mM ammonium acetate, pH 5 (30:70)	Change % and type of organic modifier
Polar organic (POM)	Acetonitrile/Methanol/acetic acid/Triethylamine (95:5:0.1:0.1)	Use other polar organic solvents or blends. Test acid:base ratios from 1:4 to 4:1. Typical acid and base concentrations are 0.01 to 1%.
Normal phase (NP) (for CYCLOBOND® DMP only)	Ethanol/Heptane (30:70)	Increase % of polar modifier. Change both solvents (e.g. Isopropanol for ethanol, test any organic solvent)

Please request our Chiral Method Development poster to see the complete Astec® CHIROBIOTIC® and CYCLOBOND® column method development screening and optimization protocols.

Astec® CYCLOBOND® Native CSPs and Derivatives

The various CYCLOBOND® phases are made by derivatization of the β - or γ -cyclodextrin molecule at the 2- or 3- position hydroxyl group. The 6-position OH is used to anchor the cyclodextrin to the silica surface. Selectivity is different among the CYCLOBOND® family members. The phases described below are available in column formats for both analytical and preparative applications. Phases marked with an asterisk (*) are among the most popular CYCLOBOND® phases.

Astec® CYCLOBOND® I 2000* Native β -Cyclodextrin

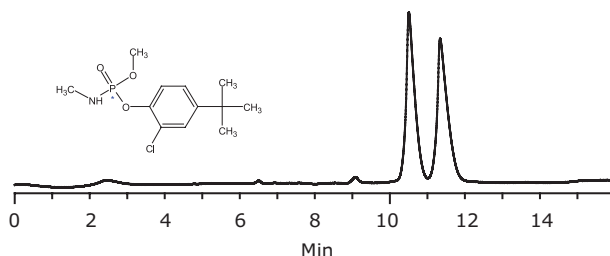
CYCLOBOND® I 2000 columns comprises β -cyclodextrin bonded by a patented process to produce a stable matrix with the cyclodextrin arranged to retain its most valuable property of forming inclusion complexes. This feature allows it to affect numerous chemical separations by selectively including into its cavity a wide variety of organic molecules. Non-inclusion-type separations are also possible with the polar organic mode for a wide variety of molecule types. Along with CYCLOBOND® I 2000 HP-RSP columns, it is among the most popular of the Astec® CYCLOBOND® column phases.

Astec® CYCLOBOND® I 2000 AC β -Cyclodextrin, peracetylated

This CSP is the peracetylated product of the native β -cyclodextrin. CYCLOBOND® I 2000 AC columns is used primarily for aromatic alcohols or amines that are chiral on the alpha or beta carbon.

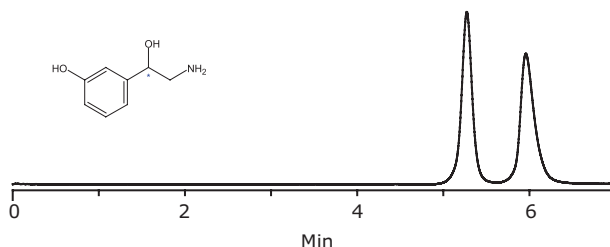
Ruelene (Cruformate) on Astec® CYCLOBOND® I 2000

column:	CYCLOBOND® I 2000, 25 cm x 4.6 mm I.D., 5 μ m (20024AST)
mobile phase:	A: acetonitrile B: acetic acid C: Triethylamine 100:0.3:0.2 (A:B:C)
flow rate:	0.6 mL/min
temp.:	25 °C
det.:	UV, 254 nm
injection:	5 μ L
sample:	Ruelene (cruformate), 1 mg/mL in acetonitrile



Norphenylephrine on Astec® CYCLOBOND® I 2000 AC

column:	CYCLOBOND® I 2000 AC, 25 cm x 4.6 mm I.D., 5 μ m (20124AST)
mobile phase:	A: methanol B: 20 mM ammonium acetate, pH 5.0 5:95 (A:B)
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 230 nm
injection:	5 μ L
sample:	norphenylephrine, 1 mg/mL in acetonitrile:water (50:50)



Astec® CYCLOBOND® I 2000 DMP*
β-Cyclodextrin, 3,5-dimethylphenyl carbamate derivative

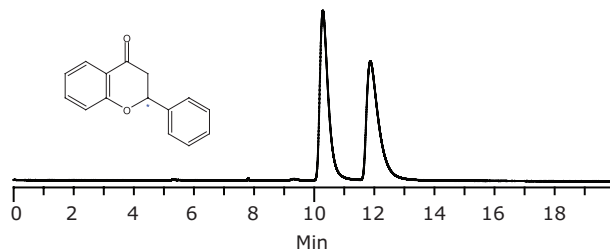
The reaction of the 3,5-dimethylphenyl isocyanate with some of the hydroxyl groups of β-cyclodextrin results in a pi-basic phase similar in character to the naphthylethyl carbamate phases. The selectivity is greater for the CYCLOBOND® I 2000 DMP column when the analyte's chiral center is part of a ring structure or is on the alpha carbon. This phase can be operated in normal phase and polar organic phase modes, in addition to typical reversed-phase mode.

Astec® CYCLOBOND® I 2000 RSP
β-Cyclodextrin, R,S-hydroxypropyl ether derivative

A general-purpose chiral stationary phase that has the added property of separating non-aromatic structures such as t-Boc amino acids, for which it is a standard methodology.

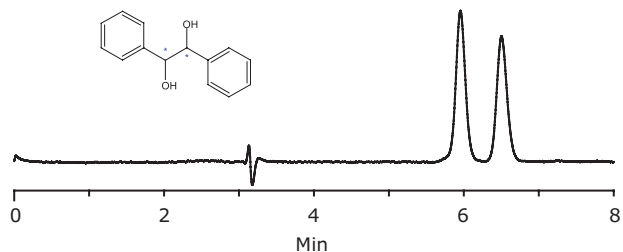
Flavanone on Astec® CYCLOBOND® I 2000 DMP

column:	CYCLOBOND® I 2000 DMP, 25 cm x 4.6 mm I.D., 5 μm (20724AST)
mobile phase:	A: isopropanol B: heptane 30:70 (A:B)
flow rate:	0.6 mL/min
temp.:	25 °C
det.:	UV, 254 nm
injection:	5 μL
sample:	flavanone, 1 mg/mL in heptane



Hydrobenzoin on Astec® CYCLOBOND® I 2000 RSP

column:	CYCLOBOND® I 2000 RSP, 25 cm x 4.6 mm I.D., 5 μm (20324AST)
mobile phase:	A: acetonitrile B: 10 mM ammonium acetate, pH 4.0 25:75 (A:B)
flow rate:	1 mL/min
temp.:	25 °C
det.:	UV, 254 nm
injection:	5 μL
sample:	hydrobenzoin, 1 mg/mL in acetonitrile:water (50:50)



Astec® CYCLOBOND® I 2000 HP-RSP*
High Performance β-Cyclodextrin, R,S-hydroxypropyl ether derivative

In the design of this chemistry, it was an objective to create a stable and reproducible phase with shorter retention times, while maintaining or improving selectivity compared with CYCLOBOND® I 2000 RSP column. After an extensive evaluation of this chemistry, that goal was attained, as well as a dramatic improvement in a number of separations. CYCLOBOND® I 2000 HP-RSP column separates by extended H-bonding capability, offers broad chiral selectivity for chiral screening, and is most beneficial for basic and neutral compounds. Along with CYCLOBOND® I 2000 column, it is among the most popular of the Astec® CYCLOBOND® phases.

Astec® CYCLOBOND® I 2000 SP
β-Cyclodextrin, S-hydroxypropyl ether derivative

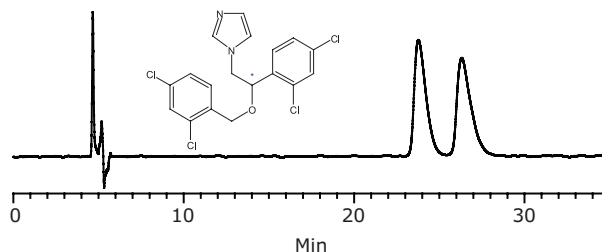
In this phase, the hydroxyl groups on the surface of the β-cyclodextrin are reacted with (S)-propylene oxide. This feature has the effect of extending hydrogen-bonding capabilities to accommodate greater distances of the chiral center from an aromatic ring structure.

Astec® CYCLOBOND® II
Native γ-Cyclodextrin

CYCLOBOND® II column is γ-cyclodextrin bonded to silica. An excellent chiral selector for multi-ring structures, this CSP is useful for isomeric compounds based on anthracene, chrysene and pyrene-type ring structures. CYCLOBOND® II column offers good selectivity and stability, and is applicable to the polar organic and reversed-phase modes. Applications include steroids, porphyrins, and FMOc amino acids.

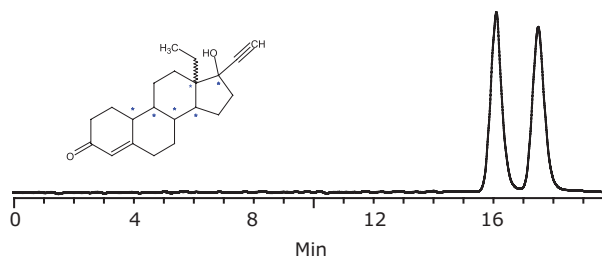
Miconazole on Astec® CYCLOBOND® I 2000 HP-RSP

column:	CYCLOBOND® I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 μm (24024AST)
mobile phase:	A: acetonitrile B: 20 mM ammonium acetate, pH 4.0 25:75 (A:B)
flow rate:	0.6 mL/min
temp.:	25 °C
det.:	UV, 230 nm
injection:	5 μL
sample:	miconazole, 1 mg/mL in acetonitrile:water (50:50)



Norgestrel on Astec® CYCLOBOND® II

column:	CYCLOBOND® II, 25 cm x 4.6 mm I.D., 5 μm (41020AST)
mobile phase:	A: water B: acetonitrile 70:30 (A:B)
flow rate:	0.8 mL/min
temp.:	22 °C
det.:	UV, 254 nm
injection:	1 μL
sample:	norgestrel, 1 mg/mL in methanol



Astec® CYCLOBOND® Columns

Other dimensions are available as a custom offering, please visit our web site or contact techservice@sial.com

CYCLOBOND® (5.0 µm)

Length (mm)		I.D. (mm)	I 2000	I 2000 AC	I 2000 SP	I 2000 RSP	I 2000 HP RSP	I 2000 DMP	II
100	x	2.1	20018AST						
150	x	2.1	20019AST						
100	x	4.6						20722AST	
150	x	4.6		20123AST			24023AST		46023AST
250	x	4.6	20024AST	20124AST	20224AST	20324AST	24024AST	20724AST	41020AST
250	x	10.0	20034AST					20734AST	
250	x	21.2						20744AST	
Guard 20	x	4.0				21103AST			
Guard Holder						21150AST			



Guard Column Holders

Guard Holders for 4 mm I.D. cartridges
(holder not required for 1 mm I.D. guards)

Cat. No.	Description
21150AST	Guard Column Holder

Astec® Cellulose DMP HPLC Columns

Astec® Cellulose DMP columns is a chiral stationary phase (CSP) comprising spherical, high-purity porous silica coated with DMPC (3,5-dimethylphenylcarbamate)-derivatized cellulose, and packed in analytical to preparative size HPLC columns. This CSP separates a wide range of chiral compounds under normal phase, polar organic, and SFC conditions, with high efficiency, high loading capacity, and excellent column lifetime. Performance is comparable to other DMPC-derivatized cellulose CSPs, but the Astec® Cellulose DMP columns are offered at a substantially lower price.

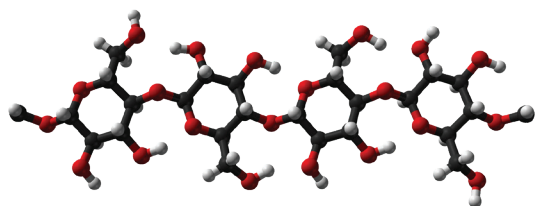
Astec® Cellulose DMP column is complementary to the other Astec® CSPs, including CHIROBIOTIC® and CYCLOBOND® product lines, and a must-have for every chiral HPLC or SFC screening protocol.

Key Features and Application Areas

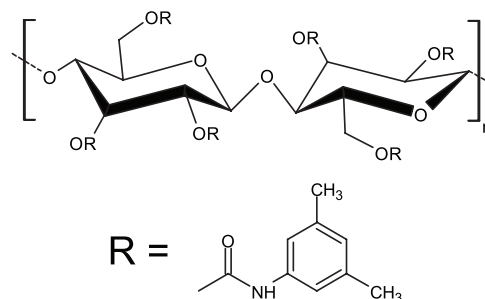
- Classic DMPC-cellulose chiral selectivity
- Efficient, rugged, reproducible, and scalable
- Low backpressure
- Ideal for chiral analysis in the pharmaceutical industry and for small analytes in chemical and environmental areas
- Routine chiral column method development screening protocols
- Approximately one-half the cost of most DMPC-cellulose columns
- Complementary selectivity to other types of CSPs
- Scalable from analytical to preparative

What is Cellulose?

The polysaccharide cellulose is a naturally occurring, optically active, linear polymer comprising hundreds to thousands of D-(+)-glucose units joined by β 1,4-glycosidic bonds. The long polysaccharide chains form rope-like bundles held together via multiple hydrogen bonds between proximate hydroxyl groups. In 1973, Hesse and Hagel described the enantioselective properties of microcrystalline cellulose triacetate (1). In the mid-1980's, Okamoto and colleagues published their work that led to the use of derivatized cellulose adsorbed onto silica as chiral HPLC stationary phases (2,3). Since then, the polysaccharides, particularly cellulose and amylose, have become the most commercially successful class of CSPs.



DMPC-Cellulose Structure



Cellulose is a linear polymer of D-(+)-glucose units linked by β 1,4-glycosidic bonds. This figure shows the cellulose tris(3,5-dimethylphenylcarbamate) derivative used in Astec® Cellulose DMP column.

How do Cellulose-Based CSPs Separate Enantiomers?

Derivatized cellulose-based CSPs, like Astec® Cellulose DMP column, owe their high enantioselectivity to the large number of chiral centers in the polysaccharide backbone and to its highly-ordered structure. The shape of the pockets formed by the intertwined chains provides chiral discrimination based on molecular shape. Derivatives at the 2, 3, and 6-position hydroxyls confer additional enantioselectivity. The dimethylphenyl carbamate derivative separates a wide range of enantiomers; the phenyl ring and carbamate groups provide π - π and hydrogen bonding interactions, respectively, with predisposed analytes.

What Makes Astec® Cellulose DMP Unique?

Astec® Cellulose DMP column is unique in offering classic DMPC-cellulose chiral selectivity, but at approximately one-half the cost of most DMPC-cellulose columns. This CSP is an ideal component of any chiral column screening portfolio, and should be investigated as an alternative to higher-priced DMPC-cellulose columns for existing methods. The cost savings are especially dramatic when comparing preparative column dimensions.

Normal Phase Chiral Separations

DMPC-derivatized cellulose is commonly run in normal phase mode. Typical normal phase mobile phases are hexane or heptane with ethanol or isopropanol as polar modifiers. The performance of Astec® Cellulose DMP column in terms of selectivity and compatibility under normal phase conditions meets or exceeds competitive phases of similar composition. The following applications show the resolution of three racemates on Astec® Cellulose DMPC column and two higher-priced competitive phases.

Comparable to Other DMPC-Cellulose Columns

For the compounds tested, Astec® Cellulose DMP columns provide similar retention and selectivity, but lower backpressure and higher efficiency, compared to competitive columns. Astec® Cellulose DMP column is not a clone of other DMPC-derivatized cellulose columns on the market, but selectivity and retention is similar enough to use it instead of these columns in chiral column HPLC and SFC screening protocols. This CSP

also should be investigated as a possible replacement for these columns in established methods. The data in the table below compares resolution, pressure, and selectivity on Astec® Cellulose DMP column and the leading competitive column. Comparison also appears in the following applications against two competitive columns.

Comparison of Astec® Cellulose DMP vs. Leading Competitor for Sample Set of Chiral Compounds

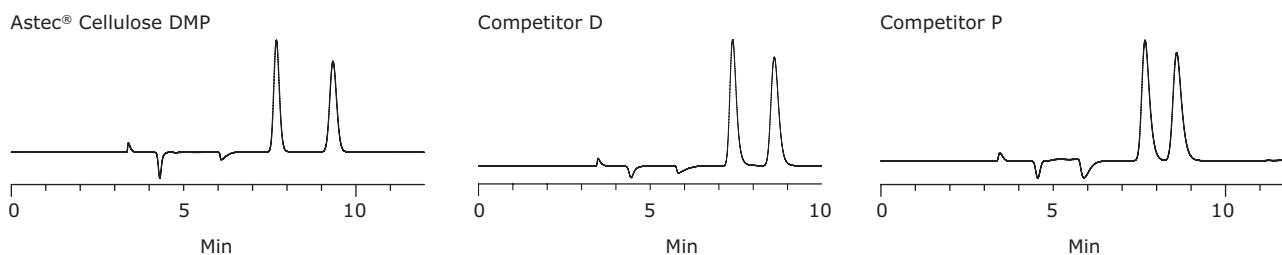
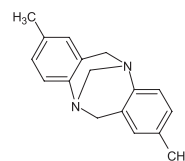
Compound	Mobile Phase*	Astec® Cellulose DMP				Competitor D			
		Pressure (bar)	Selectivity	N (Peak 1)	Resolution	Pressure (Bar)	Selectivity	N (Peak 1)	Resolution
Alprenolol	A	16	3.0	9,528	12.8	19	1.4	5,963	3.5
Atropine	A	16	1.4	5,768	4.9	19	1.7	3,666	6.1
Benzoin	B	16	1.4	7,690	6.2	19	1.6	6,217	7.1
Diperodon	C	21	3.9	5,915	14.4	26	4.0	4,846	12.8
Etodolac	B	16	2.6	6,323	10.2	19	2.8	5,568	10.1
Hydroxyzine	A	16	1.2	5,477	1.9	19	1.2	4,173	2.1
Ketamine	A	16	1.2	9,506	2.4	19	1.2	8,172	2.3
Metoprolol	A	16	2.3	7,208	7.0	19	2.6	5,343	6.0
Mianserin	A	16	1.2	6,936	1.9	19	1.2	6,078	1.6
Proglumide	B	16	1.8	3,672	5.3	19	2.2	2,963	5.5
<i>trans</i> -Stilbene Oxide (TSO)	D	16	1.9	13,753	9.9	19	1.9	11,871	9.1
Tröger's Base	A	16	1.4	9,398	4.8	19	1.3	7,375	3.0

columns: 15 cm x 4.6 mm I.D., 5 µm, flow rate: 0.5 mL/min temp.: 25 °C (Note: Separations not optimized on either column)

*mobile phase: A: 10:90:0.1, Isopropanol:heptane:Diethylamine
 B: 10:90:0.1, Isopropanol:heptane:Trifluoroacetic acid
 C: 0.1% w/v ammonium formate in methanol
 D: 10:90, Isopropanol:heptane

Tröger's Base - Competitive Comparison

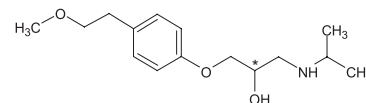
columns:	15 cm x 4.6 mm I.D., 5 µm
mobile phase:	10:90:0.1, Isopropanol:heptane:Diethylamine
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 230 nm
inj.:	2 µL
sample:	Tröger's Base (2 mg/mL)



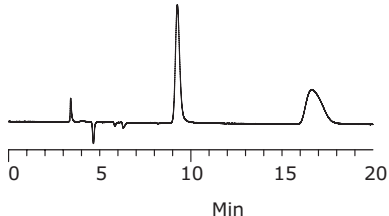
Metoprolol - Competitive Comparison

Conditions same as previous application except:

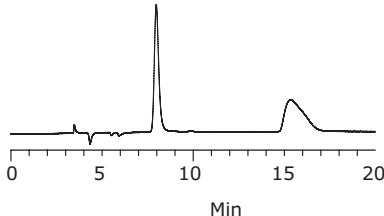
sample: metoprolol



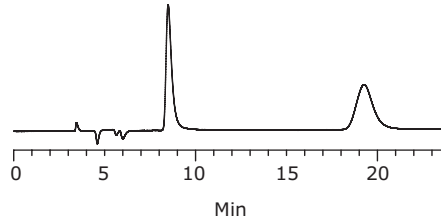
Astec® Cellulose DMP



Competitor D



Competitor P

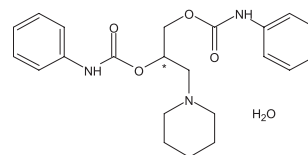


Diperodon - Competitive Comparison

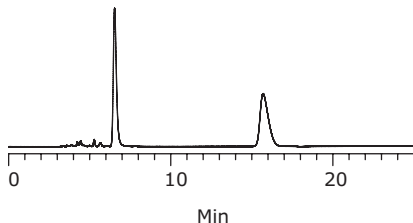
Conditions same as previous application except:

mobile phase: 0.1% ammonium formate in methanol

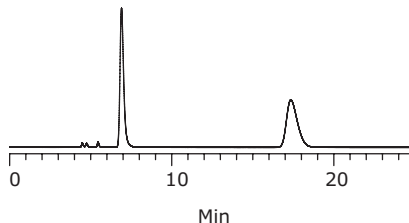
sample: diperodon



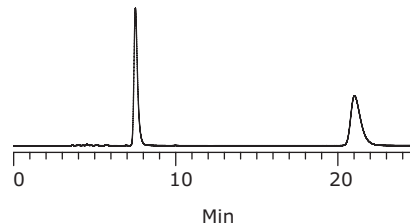
Astec® Cellulose DMP



Competitor D



Competitor P



Normal Phase Applications

Alprenolol

column: Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)

mobile phase: 10:90:0.1, Isopropanol:heptane:Diethylamine

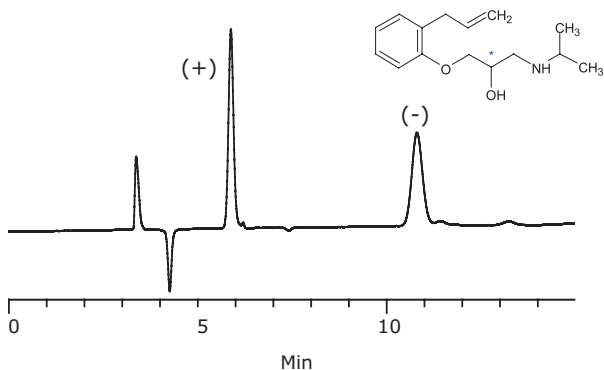
flow rate: 0.5 mL/min

temp.: 25 °C

det.: UV, 254 nm

inj.: 2 µL

sample: alprenolol (2 mg/mL in mobile phase)



Atropine

column: Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)

mobile phase: 10:90:0.1, Isopropanol:heptane:Diethylamine

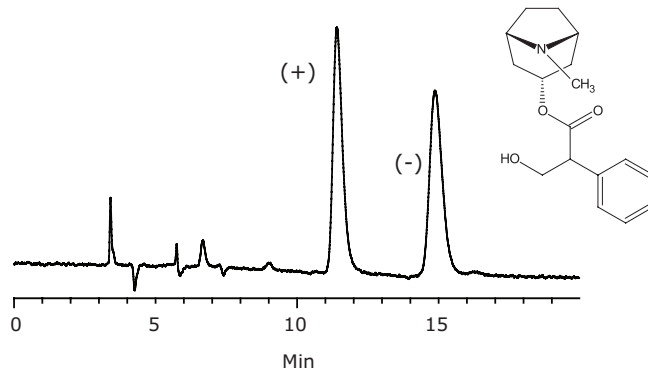
flow rate: 0.5 mL/min

temp.: 25 °C

det.: UV, 254 nm

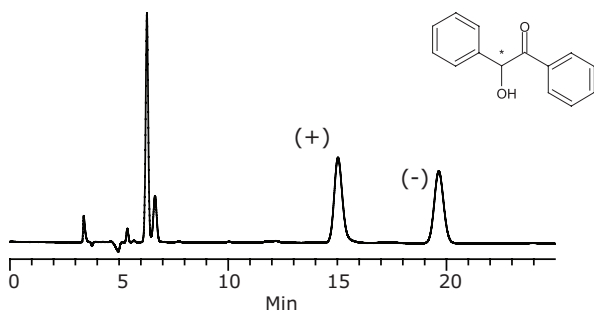
inj.: 2 µL

sample: atropine (2 mg/mL in mobile phase)



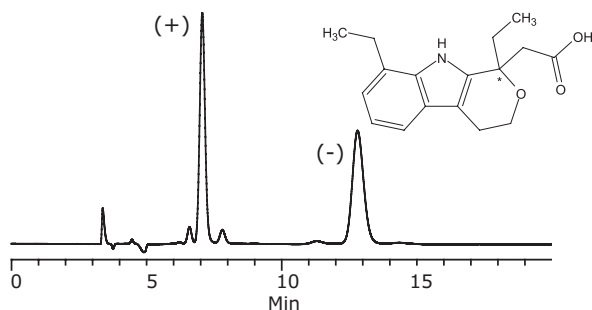
Benzoin

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10:90:0.1, Isopropanol:heptane:Trifluoroacetic acid
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 254 nm
inj.:	2 µL
sample:	benzoin (2 mg/mL in mobile phase)



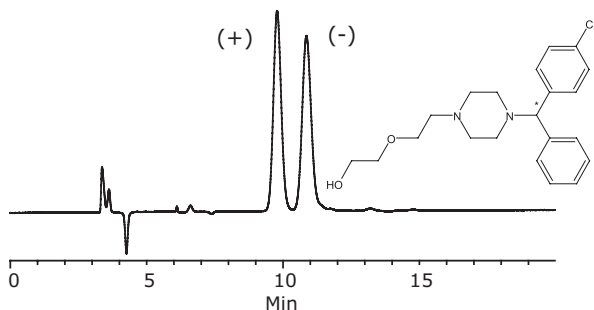
Etodolac

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10:90:0.1, Isopropanol:heptane:Trifluoroacetic acid
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 254 nm
inj.:	2 µL
sample:	etodolac (2 mg/mL in mobile phase)



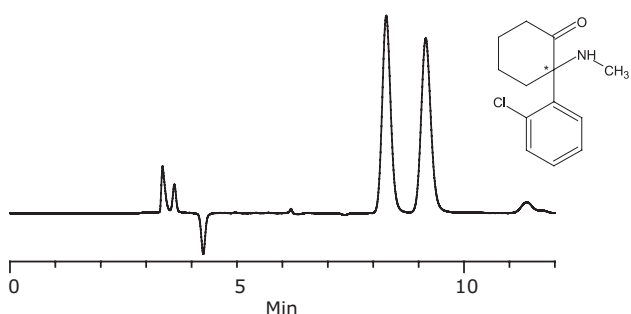
Hydroxyzine

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10:90:0.1, Isopropanol:heptane:Diethylamine
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 254 nm
inj.:	2 µL
sample:	hydroxyzine (2 mg/mL in mobile phase)



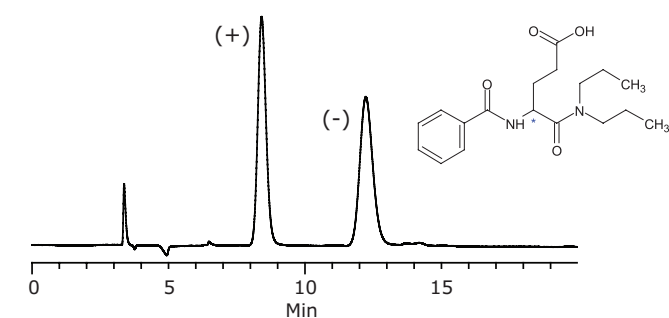
Ketamine

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10:90:0.1, Isopropanol:heptane:Diethylamine
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 254 nm
inj.:	2 µL
sample:	ketamine (2 mg/mL in mobile phase)



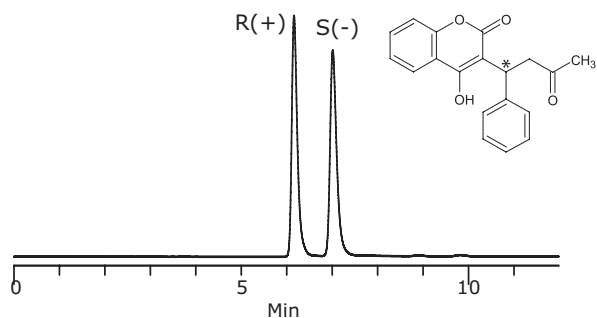
Proglimide

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10:90:0.1, Isopropanol:heptane:Trifluoroacetic acid
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 254 nm
inj.:	2 µL
sample:	proglumide (2 mg/mL in mobile phase)



Warfarin

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	100:0.2:0.1, Methanol:acetic acid:Triethylamine
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 278 nm
inj.:	2 µL
sample:	warfarin (2 mg/mL in mobile phase)



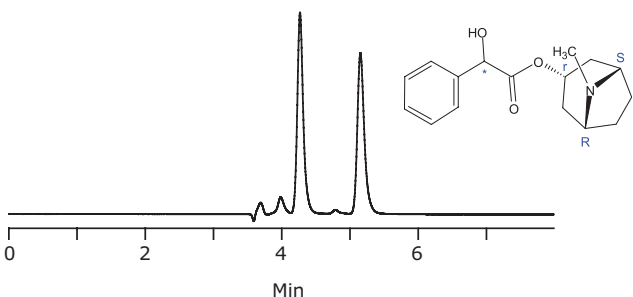
Polar Organic Mode Chiral Separations

Polar organic mode (POM) mobile phases comprise methanol or acetonitrile, often with acids, bases, or salts added to control peak shape and retention of certain sensitive analytes. The benefit of POM is realized when dealing with compounds that are poorly soluble in non-polar normal phase mobile phases. For preparative chiral applications, solubility is especially important; analyte concentration per injection influences the throughput. Astec® Cellulose DMP column operates in POM to permit choice of mobile phase based on analyte solubility. A few of the racemates resolved on Astec® Cellulose DMP column with different POM mobile phases appear in the table below and in the following applications.

Not only does Astec® Cellulose DMP column provide excellent resolution in POM and NP modes, it also is rugged enough to hold up to repeated NP-POM-NP-POM cycles without loss of performance. The figure that follows demonstrates the same Astec® Cellulose DMP column used alternately in normal phase and POM mobile phases. Performance values appear in the figure.

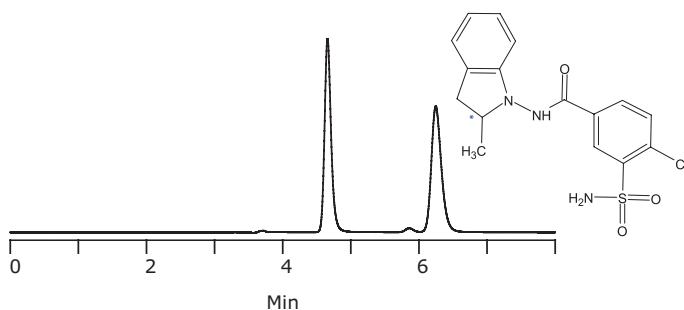
Homatropine

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	0.1% v/v Diethylamine in methanol
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 230 nm
inj.:	2 µL

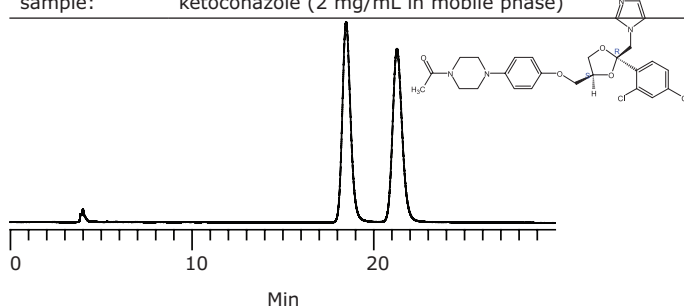


Indapamide

column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	0.1% w/v ammonium formate in methanol
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 230 nm
inj.:	2 µL
sample:	indapamide (2 mg/mL in mobile phase)



sample:	homatropine (2 mg/mL in mobile phase)
Ketoconazole	
column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	acetonitrile
flow rate:	0.5 mL/min
temp.:	25 °C
det.:	UV, 230 nm
inj.:	2 µL
sample:	ketoconazole (2 mg/mL in mobile phase)



Performance of Astec® Cellulose DMP in Polar Organic Mode (POM)

Mode:	POM - Methanol		POM - LC-MS Conditions		POM - Acetonitrile (no additives)	
Mobile Phase:	0.1% v/v DEA in Methanol		0.1% w/v Ammonium Formate in Methanol		100% Acetonitrile	
Compound	t _R Peak 1 (min)	Selectivity	t _R Peak 1 (min)	Selectivity	t _R Peak 1 (min)	Selectivity
Diperodon	6.63	3.94	5.86	3.92	9.76	8.08
Homatropine	4.27	2.02	3.68	2.00		
Indapamide	4.71	2.32	4.66	2.26		
Ketoconazole					18.47	1.19
Mianserin	6.06	1.33	6.15	1.43	5.28	1.26
Tröger's Base	7.37	1.14	7.41	1.17		
Warfarin*	6.16	1.31				

Column: Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST), flow rate: 0.5 mL/min, temp.: 25 °C

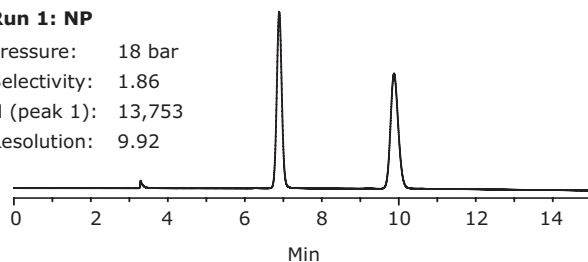
*Warfarin mobile phase: 100:0.2:0.1, methanol:acetic acid:Triethylamine

Stable Performance After Repeated NP-POM Cycles

columns:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase (normal phase):	10:90, Isopropanol:heptane
mobile phase (POM):	0.1% w/v ammonium formate in methanol
flow rate:	0.5 mL/min
temp.:	25 °C
sample:	TSO (normal phase) or mianserin (POM)
det.:	UV, 254 nm
inj.:	2 µL

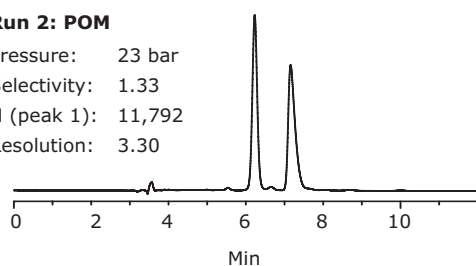
Run 1: NP

Pressure: 18 bar
 Selectivity: 1.86
 N (peak 1): 13,753
 Resolution: 9.92



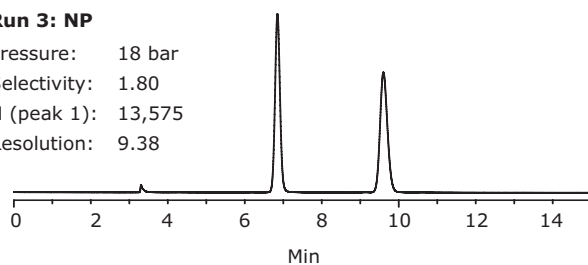
Run 2: POM

Pressure: 23 bar
 Selectivity: 1.33
 N (peak 1): 11,792
 Resolution: 3.30



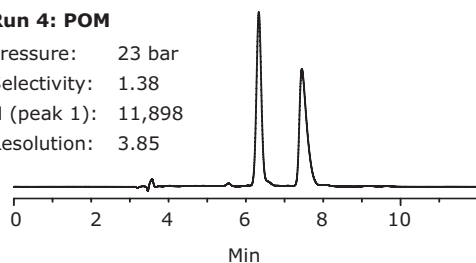
Run 3: NP

Pressure: 18 bar
 Selectivity: 1.80
 N (peak 1): 13,575
 Resolution: 9.38



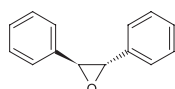
Run 4: POM

Pressure: 23 bar
 Selectivity: 1.38
 N (peak 1): 11,898
 Resolution: 3.85

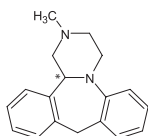


Analyte Structure:

trans-Stilbene Oxide



Mianserin

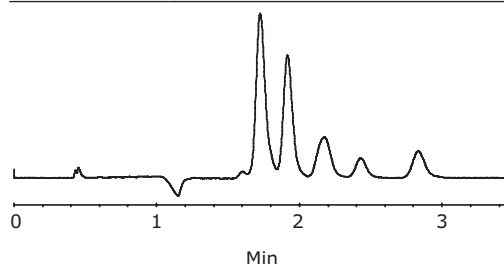


SFC Chiral Separations

SFC (supercritical fluid chromatography) is gaining in popularity, especially for chiral separations, due to its speed advantages over HPLC. The carbon dioxide is readily removed from the eluate, which makes it ideal for prep. DMPC-derivatized cellulose is widely used for chiral separations by SFC, both analytical and prep. The Astec® Cellulose DMP column works well in SFC mode, providing rapid separations with excellent selectivity. For example, the following application shows the SFC separation of a mixture of six diastereomers of a single compound in less than four minutes on Astec® Cellulose DMP column. As a testament to its utility as a screening tool, the durability of Astec® Cellulose DMP column permits rapid (ballistic) gradients of methanol, ethanol or isopropanol in carbon dioxide with long column lifetime and low backpressure, but without significant column bleed.

Rapid SFC Separations

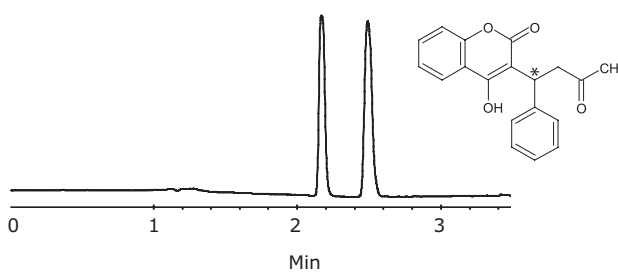
column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10% ethanol in carbon dioxide
flow rate:	3 mL/min
temp.:	35 °C
pressure:	100 bar
det.:	UV, 220 nm
inj.:	5 µL
sample:	six diastereomers of a single compound (proprietary drug substance)



Stability and Resolution under Ballistic SFC Gradients

Conditions same as previous application except:

gradient:	5-65% methanol in carbon dioxide; hold 1 min
flow rate:	4 mL/min
sample:	warfarin, 2 mg/mL



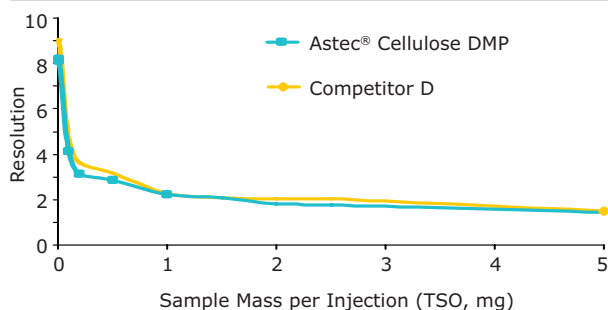
SFC data for these applications kindly provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.

Ideal for Preparative Applications

A characteristic of the polysaccharide-based CSPs that has contributed to their popularity is their utility for preparative applications. Although there are many factors to consider in a preparative scale up, the particle contribution comes primarily from the amount of available stationary phase and to what degree it resolves the enantiomers. When the Astec® Cellulose DMP columns were designed, the goal was to achieve the high sample loading and throughput that chiral chromatographers have come to expect. The sample loading (mg per injection) of Astec® Cellulose DMP column is comparable to Competitor D. An example of the scale-up of an analytical separation (4.6 mm I.D. column) to a preparative scale (21.2 mm I.D.) is shown in the below application for the anti-Alzheimer's drug BAY 73-6691.

Loading Capacity

columns:	15 cm x 4.6 mm I.D., 5 µm
mobile phase:	10:90, Isopropanol:hexane
flow rate:	1 mL/min
temp.:	28 °C
det.:	UV, 210 nm
inj.:	100 µL
sample:	<i>trans</i> -stilbene oxide (TSO), 0.05 – 50 mg/mL



Scale-Up Example

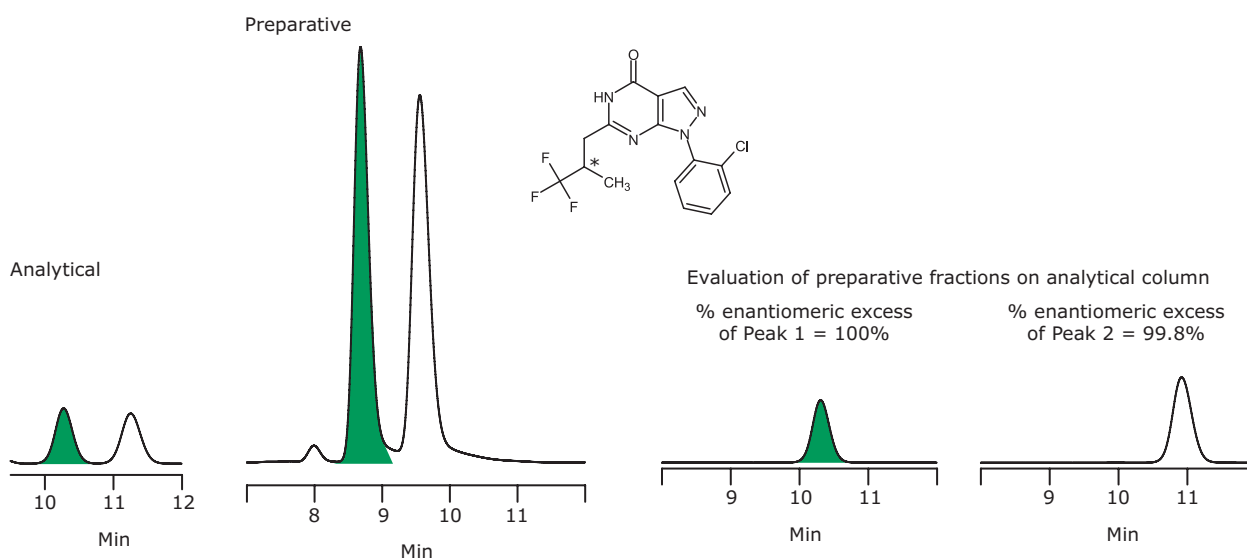
columns:	Astec® Cellulose DMP, 5 µm
mobile phase:	80:10:10, heptane:methyl <i>tert</i> -butyl ether (MTBE):ethanol
temp.:	25 °C
det.:	UV, 230 nm
sample:	BAY 73-6691 in mobile phase

Analytical:

dimensions:	15 cm x 4.6 mm I.D. (51098AST)
flow rate:	0.5 mL/min
inj.:	10 µL (2 mg/mL)

Preparative:

dimensions:	25 cm x 21.2 mm I.D. (51103AST)
flow rate:	13 mL/min
inj.:	5000 µL (3.3 mg/mL)



76 stacked injections were made, processing a total of 10 grams of racemic material

Rugged Columns, Stable Phase Chemistry, No Memory Effect

Although Astec® Cellulose DMP column is a coated phase, the DMPC-cellulose is held securely onto the silica surface. The below data set shows chromatograms after long-term use. The column was exposed to over 13 liters of mobile phase, nearly 2,000 injections and 10 days of continuous operation without significant change in any chromatographic parameter. The Astec® Cellulose DMP column has little memory effect when switching between mobile phase systems. This stability and lack of memory effect make the Astec® Cellulose DMP columns even more of a value; not only are they considerably less expensive than competitive phases, their ruggedness means they maintain their high performance for long-term operation. (Avoid using polar, aprotic, and halogenated solvents.)

Stability Demonstration

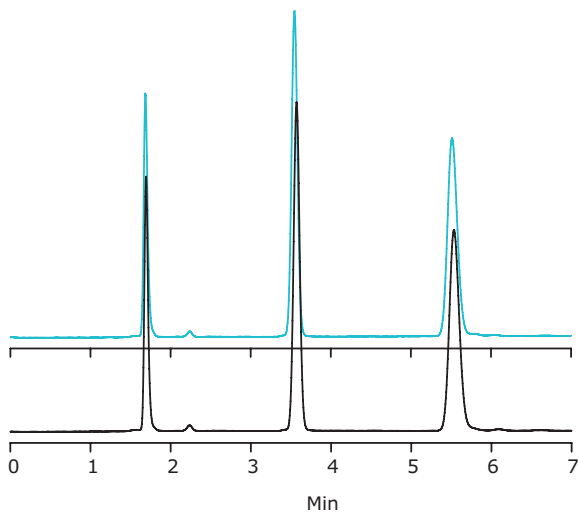
column:	Astec® Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	10:90, Isopropanol:heptane
flow rate:	1 mL/min
temp.:	28 °C
det.:	UV, 210 nm
inj.:	2 µL
sample:	TSO, 1 mg/mL

Initial performance

N = 10,800; AF10 = 0.94;
Rs = 11.1; Press. = 26 bar

After ~5000 column volumes (~13 L of mobile phase)

N = 10,400; AF10 = 0.95;
Rs = 10.8; Press. = 27 bar

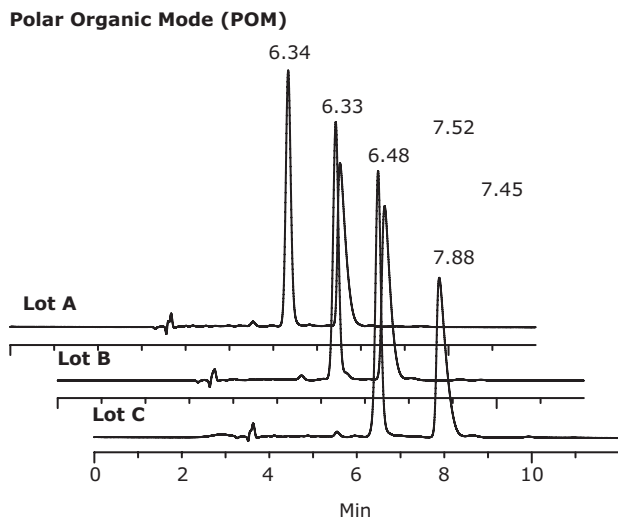
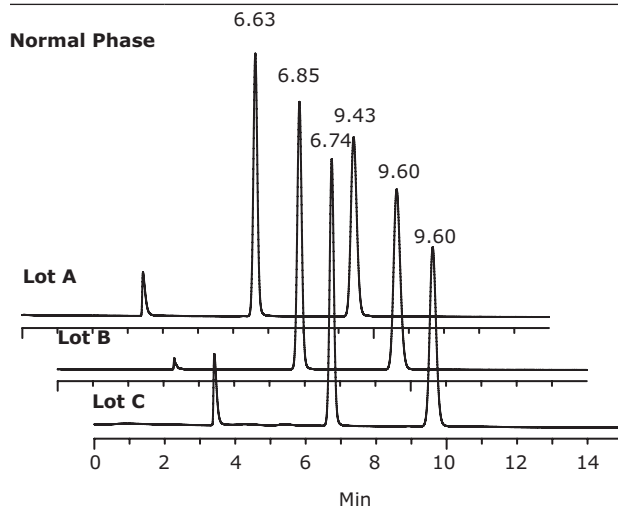


Reproducibility for Reliable Methods

No separation is useful if it is not reproducible. Astec® Cellulose DMP column was designed to have the reproducibility required for method validation. The example below shows columns from three different production lots of Astec® Cellulose DMP columns in both normal phase and polar organic modes.

Reproducibility Demonstration

column:	Astec® Cellulose DMP column, 15 cm x 4.6 mm I.D., 5 µm (51098AST)
mobile phase:	(normal phase): 10:90, Isopropanol:heptane (POM): 0.1% w/v ammonium formate in methanol
flow rate:	0.5 mL/min
temp.:	25 °C
sample:	TSO (normal phase) or mianserin (POM)
det.:	UV, 230 nm
inj.:	2 µL



Complementary to the Other Astec® CSPs

Other Astec® HPLC CSPs include CHIROBIOTIC®, CYCLOBOND®, CLC-L, and CLC-D. Astec® CHIRALDEX and Supelco® DEX™ columns are the market leaders for chiral GC separations. The HPLC CSPs are complementary to each other in terms of selectivity and mobile phase compatibility. This fact means:

- It is likely that at least one Astec® CSP will give the necessary selectivity. Incorporating Astec® Cellulose DMP, CHIROBIOTIC® and CYCLOBOND® in the HPLC or SFC screening protocol will give at least 90% success rate.
- Multiple Astec® CSPs may provide the necessary enantioselectivity, but one may operate in a




preferred mobile phase system, one that is more compatible with the detection mode, or provides better analyte solubility or shorter retention time, or many other considerations. For example, polar ionic CHIROBIOTIC® mobile phases are ideal for LC/ESI-MS.

- Different CSPs may provide reversal of elution order, a useful attribute for prep and for low-level detection of the presence of an unwanted enantiomer in large excess of the opposite enantiomer (trace analysis).

The wide choice of CSPs in the Astec® line means they cover many different areas of interest within chiral chromatography. Some of these areas are captured in the below table.

Techniques, Applications, and Fields of Use for Astec® Chiral Phases

	Astec® Cellulose DMP	Astec® CHIROBIOTIC®	Astec® CYCLOBOND®	Astec® CLC	Astec® CHIRALDEX	Supelco® DEX™
Routine Chiral Column Screening	Highly suitable	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Highly suitable	Highly suitable
Normal Phase HPLC	Highly suitable	Marginally suitable, or limited to specific applications	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
SFC	Highly suitable	Marginally suitable, or limited to specific applications	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Reversed-Phase HPLC	Not suitable nor recommended	Highly suitable	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended
Hydrophilic Interaction HPLC (HILIC)	Not suitable nor recommended	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Polar Organic Mode (HPLC)	Highly suitable	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Polar Ionic Mode (HPLC)	Not suitable nor recommended	Highly suitable	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Ligand Exchange Mode HPLC	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended	Highly suitable	Not suitable nor recommended	Not suitable nor recommended
Gas Chromatography (GC)	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended	Highly suitable	Highly suitable
Prep (LC and/or SFC)	Highly suitable	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Polar/Ionic Analytes	Marginally suitable, or limited to specific applications	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Amino Acids, Peptides	Not suitable nor recommended	Highly suitable	Not suitable nor recommended	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended
Non-Aromatic Organic Acids	Not suitable nor recommended	Not suitable nor recommended	Marginally suitable, or limited to specific applications	Highly suitable	Not suitable nor recommended	Not suitable nor recommended
Mass Spec (LC/ESI)	Marginally suitable, or limited to specific applications	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Mass Spec (LC/APCI)	Highly suitable	Highly suitable	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended
Mass Spec (GC/MS)	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended	Not suitable nor recommended	Highly suitable	Highly suitable
Bioanalysis (drugs in biological fluids)	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Marginally suitable, or limited to specific applications	Not suitable nor recommended	Highly suitable	Highly suitable

	Highly suitable
	Marginally suitable, or limited to specific applications
	Not suitable nor recommended

Ordering Information

Cellulose DMP (5.0 μm)

Length (mm)		I.D. (mm)	SKU
150	x	2.1	51100AST
100	x	4.6	51097AST
150	x	4.6	51098AST
250	x	4.6	51099AST
Guard 20	x	2.1	51104AST
Guard 20	x	4.0	51106AST
Kit 20	x	2.1	51105AST

References

1. Hesse, G.; Hagel, R. *Chromatographia* 1973, 6(6), 277 – 280.
2. Okamoto, Y.; Kawashima, M.; Yamamoto, K.; Hadata, K. *Chem. Lett. (The Journal of the Chemical Society of Japan)* 1984, 739 – 742.
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Astec® CLC-L and CLC-D HPLC Columns

Copper Ligand Exchange HPLC Columns for

Chiral Separation of Acids and Amines

Astec® CLC columns use the copper ligand concept described by Davankov to effect enantiomer separation (1). The method uses a small, chiral bidentate ligand attached to the silica surface and a copper sulphate-containing mobile phase. The copper ions coordinate with the chiral selector on the stationary phase and carboxylic acid functional groups on the analytes to form transient diastereomeric complexes in solution. The technique also has the advantage of giving small acids with no UV chromophore a strong 254 nm signal.

Astec® CLC columns are ideal for analysis of alpha-hydroxy acids, like lactic, malic, tartaric and mandelic acids, amino acids, other amines and bi-functional racemates, like amino alcohols. Two versions of the column provide elution order reversal. On the Astec® CLC-D column, the L enantiomer generally elutes before D, with the exception of tartaric acid. The reverse is true on the Astec® CLC-L column where D elutes before L. Proline and aspartic acid are particularly suited for low-level detection on the CLC column since the copper complex is detected at 254 nm UV. Both can be resolved on the Astec® CLC-D or CLC-L columns in 5 mM copper sulfate with the usual reversal of elution order from the CLC-D to CLC-L columns. In theory, any analyte that can complete the coordination with the copper ion can be resolved.

Features:

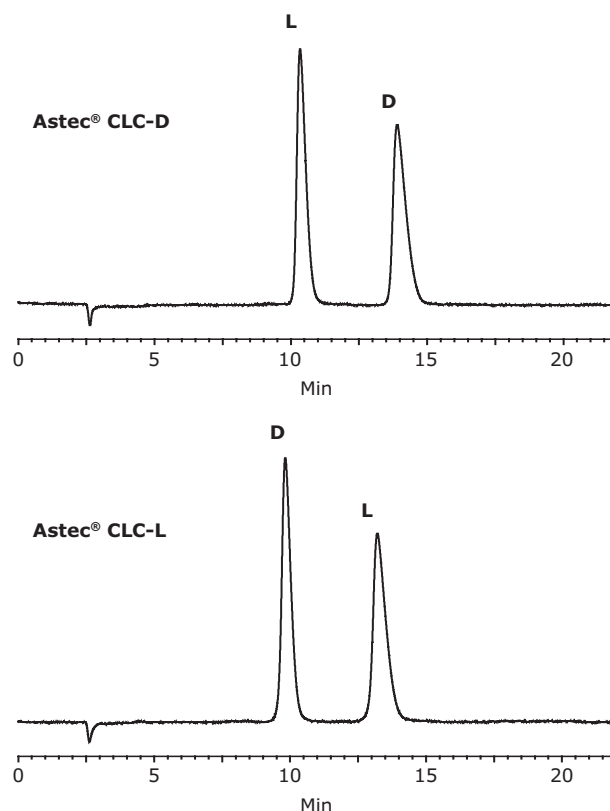
- Separates α -hydroxy carboxylic acids, amino acids and other α -bifunctional compounds
- High selectivity with simple mobile phases
- Copper complex gives strong UV 254 nm signal
- Simple reversal of elution order, CLC-L vs. CLC-D

Properties of CLC-L and CLC-D:

- Bonded phase: Chiral bidentate ligand (L and D forms)
- Operating pH range: 3 - 6 (adjust pH of the 5 mM copper sulfate mobile phase with acetic acid)
- Particle type: High-purity spherical silica
- Particle diameter: 5 μm
- Pore size: 100 \AA

Reversal of Elution Order of Lactic Acid Enantiomers on Astec® CLC-L and CLC-D

columns:	Astec® CLC-D (53023AST) and Astec® CLC-L (53123AST), both 15 cm x 4.6 mm I.D., 5 μm
mobile phase:	5 mM Copper sulfate
flow rate:	1.0 mL/min
temp.:	25 °C
det.:	UV at 254 nm
injection:	5 μL
sample:	D,L-lactic acid (3 mg/mL)



Method Development and Optimization Protocols for Astec® CLC Columns

For the ligand exchange process to occur, the mobile phase must contain 1 to 10 mM copper sulfate (CuSO₄). The recommended starting mobile phase is 20% methanol in 5 mM copper sulfate.

Optimization Parameters and Guidelines:

Parameter	Relationship	Range
Copper sulfate concentration	Retention is inversely proportional to the copper sulfate concentration	1 to 10 mM
% Organic modifier	Retention is inversely proportional to the % of organic modifier	Methanol – up to 30% Ethanol – up to 20% Isopropanol – up to 15%
Temperature	Retention is inversely proportional to temperature	5 – 40 °C
pH	Retention is proportional to the pH	pH 3 to pH 6

Part of the Astec® Family of Chiral HPLC, GC and SFC Columns:

- **Astec® CHIROBIOTIC®** – Macrocyclic Glycopeptides for Chiral HPLC
- **Astec® CYCLOBOND®** – Native and Derivatized Cyclodextrins for Chiral HPLC
- **Astec® P-CAP™** – Polycyclic Amine Polymers for Chiral HPLC
- **Astec® Cellulose DMP** – Rugged and Economical Derivatized Cellulose for Chiral HPLC and SFC
- **Astec® CLC** – Ligand Exchange for Chiral HPLC
- **Astec® CHIRALDEX and Supelco® DEX™** – Derivatized Cyclodextrins for Chiral GC

Product Listing

Description	Cat. No.
Astec® CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles	53023AST
Astec® CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles	53123AST

1. Davankov, V. A.; Rogozhin, S. V. Ligand chromatography as a novel method for the investigation of mixed complexes: Stereoselective effects in α-amino acid copper(II) complexes. J. Chrom. A. 1971, 60, 284-312.

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