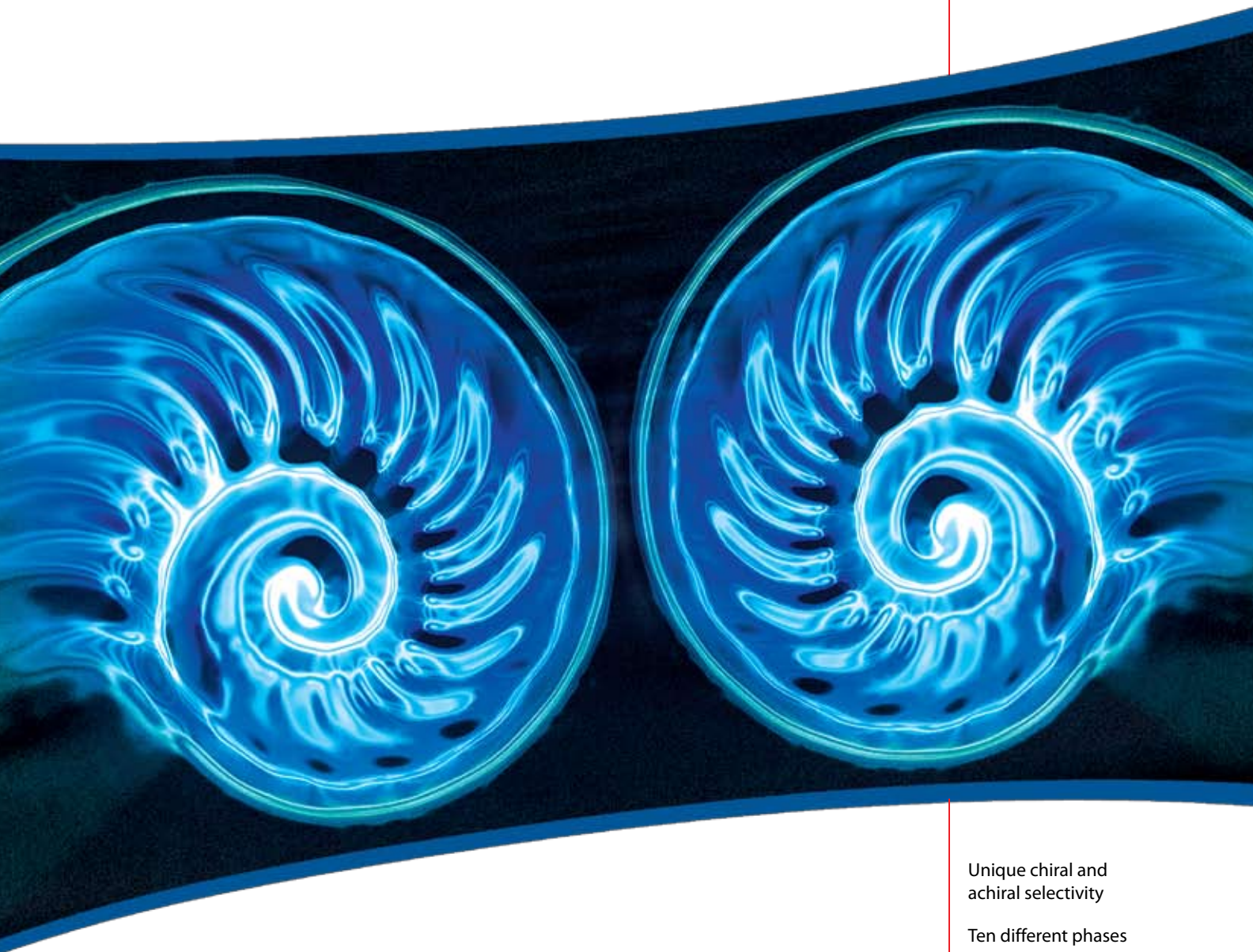


Astec CYCLOBOND

Bonded Cyclodextrin-based
Chiral HPLC Phases



Unique chiral and
achiral selectivity

Ten different phases

RP, NP, LC-MS, and
SFC compatible

No solvent or additive
memory effects

Astec CYCLOBOND

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, macrocyclic glycopeptide-based Astec CHIROBIOTIC® CSPs, and the amine copolymer-based Astec P-CAP™ and Astec P-CAP-DP.

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

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 seven 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 and the peracetylated derivative bonded to high-purity silica gel
- Excellent chiral selectors for multi-ring structures, such as those based on anthracene, chrysene, or pyrene

Table 1. 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 DM	Beta (β)	Dimethyl
Astec CYCLOBOND I 2000 DMP	Beta (β)	3,5-Dimethylphenylcarbamate
Astec CYCLOBOND I 2000 DNP	Beta (β)	2,6-Dinitro-4-trifluoromethyl phenyl ether
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)
Astec CYCLOBOND II AC	Gamma (γ)	Acetyl

TRADEMARKS: CHIROBIOTIC, CYCLOBOND, and P-CAP — Sigma-Aldrich Biotechnology, LP

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 **Figure 1**). Three highly-characterized cyclodextrins are alpha (α), beta (β), and gamma (γ) cyclodextrin, which contain six, seven, and eight glucose units, respectively (see **Table 2**). 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 very sensitive to structural differences. When the analyte possesses an aromatic

Figure 1. Proposed Structure of Cyclodextrin Molecules

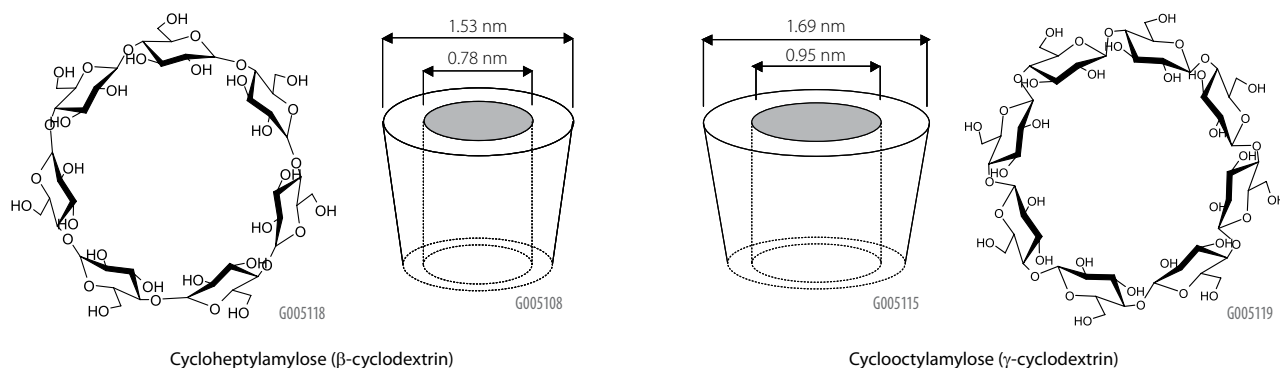


Table 2. Properties of Cyclodextrins

Cyclodextrin	Chemical Name	Glucose Units	Stereogenic Centers	Cavity Size (nm)
Alpha (α)*	Cyclohexylamylose	6	30	0.57
Beta (β)	Cycloheptamylose	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 Figure 2a. 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 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 Figure 2b.

Figure 2a: 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.



Figure 2b: 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

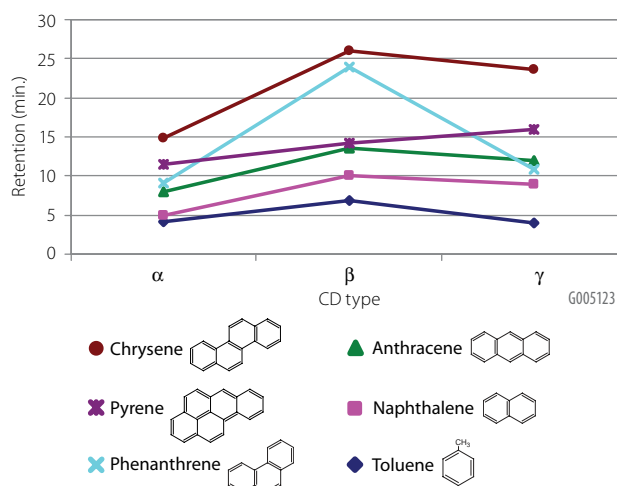
Certain analyte functional groups have a strong affinity for the cyclodextrin cavity. Other polar groups strongly hydrogen bond to the high-density hydroxyl surface of the native cyclodextrin. Derivatization of the cyclodextrin molecule at the 2- and 3-position hydroxyl groups affects selectivity and can be leveraged to alter the extent to which inclusion complexing occurs. For example, derivatized cyclodextrins, such as CYCLOBOND I 2000 DNP (dinitrophenyl) and 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

Figure 3 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, see Figure 12). The β -cyclodextrin (CYCLOBOND I 2000 series) is a better option for enantiomers of naphthalene-like structures or singly-substituted aromatic ring structures.

Figure 3. 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 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 and its derivatives. 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 are compounds with hydrogen-bonding functional groups off a ring, cis/trans and positional isomers (e.g. **Figure 4**), closely-related achiral molecules, and derivatized chiral amino acids.

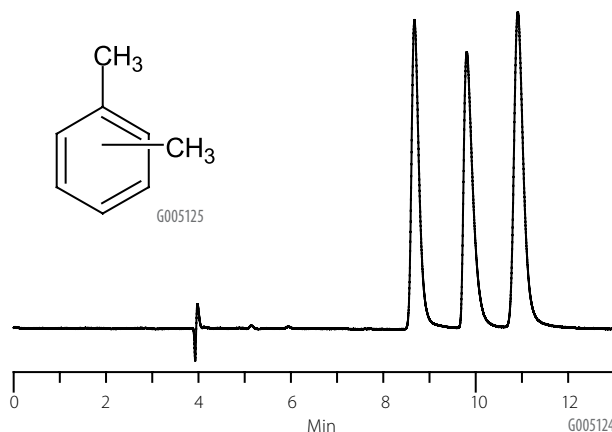
Mobile Phases for Astec CYCLOBOND CSPs

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 CO₂ containing typical co-solvents (methanol, ethanol, IPA) and additives (TFA, TEA, DEA, etc.). Favors hydrogen bonding or π - π interaction on derivatized cyclodextrins (CYCLOBOND DMP and DNP 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.

Figure 4. Positional Isomers (Xylenes) on Astec CYCLOBOND I 2000

column: CYCLOBOND I 2000, 25 cm x 4.6 mm I.D.,
5 μ m particles (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. Their unique selectivity makes them complementary to other CSPs, and may provide the extra resolution needed to separate the target enantiomers. **Table 3** outlines our recommended method development screening protocol for CYCLOBOND columns in the different mobile phase systems.

Table 3. Astec CYCLOBOND Screening Protocol

Mobile Phase System	Starting Composition	Optimization
Reversed-phase (RP)	CH ₃ OH or CH ₃ CN/20 mM ammonium acetate, pH 5 (30:70)	Change % and type of organic modifier
Polar organic (POM)	CH ₃ CN/CH ₃ OH/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 DNP and DMP only)	Ethanol/Heptane (30:70)	Increase % of polar modifier. Change both solvents (e.g. IPA for ethanol, test any organic solvent)

Please request our Chiral Method Development poster (T409107) 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, and are included in our Method Development Kit (20005AST).

Astec CYCLOBOND I 2000*

Native β -Cyclodextrin

CYCLOBOND I 2000 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 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, it is among the most popular of the Astec CYCLOBOND phases.

Astec CYCLOBOND I 2000 AC

β -Cyclodextrin, peracetylated

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

Astec CYCLOBOND I 2000 DM

β -Cyclodextrin, dimethylated

This is the dimethylated product of the native β -cyclodextrin. CYCLOBOND I 2000 DM separates a wide variety of structural and geometric isomers as well as a group of enantiomers not resolved on CYCLOBOND I 2000. This phase operates only in the reversed-phase mode by steric bulk as the main mechanism.

Figure 5. Ruelene (Cruformate) on Astec CYCLOBOND I 2000

column: CYCLOBOND I 2000, 5 μ m particles (20024AST)
mobile phase A: acetonitrile
mobile phase B: acetic acid
mobile phase C: Triethylamine
mobile phase ratio: 100:0.3:0.2 (A:B:C)
flow rate: 0.6 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 254 nm
injection: 5 μ L
sample: Ruelene (cruformate), 1 mg/mL in acetonitrile

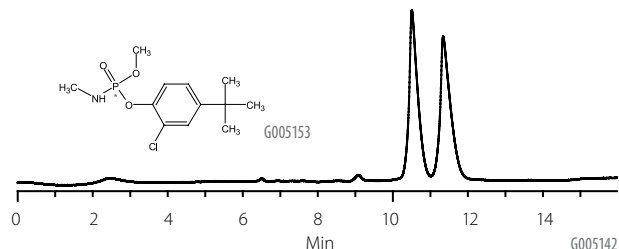


Figure 6. Norphenylephrine on Astec CYCLOBOND I 2000 AC

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

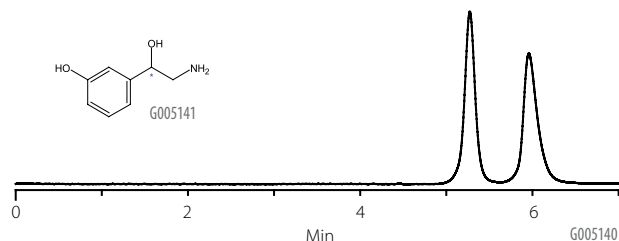


Figure 7. Coumachlor on Astec CYCLOBOND I 2000 DM

column: CYCLOBOND I 2000 DM, 25 cm x 4.6 mm I.D., 5 μ m particles (20924AST)
mobile phase A: acetonitrile
mobile phase B: 20 mM ammonium acetate, pH 2.9
mobile phase ratio: 20:80 (A:B)
flow rate: 0.8 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 230 nm
injection: 3 μ L
sample: coumachlor, 1 mg/mL in acetonitrile:water (50:50)

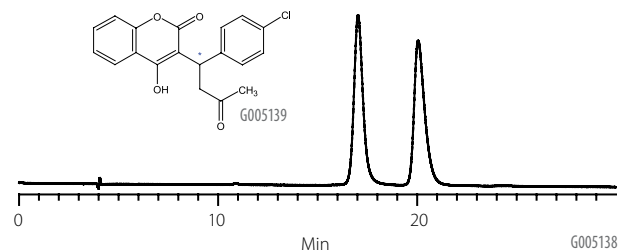




Figure 8. Flavanone on Astec CYCLOBOND I 2000 DMP

column: CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D.,
5 μ m particles (20724AST)
mobile phase A: isopropanol
mobile phase B: heptane
mobile phase ratio: 30:70 (A:B)
flow rate: 0.6 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 254 nm
injection: 5 μ L
sample: flavanone, 1 mg/mL in heptane

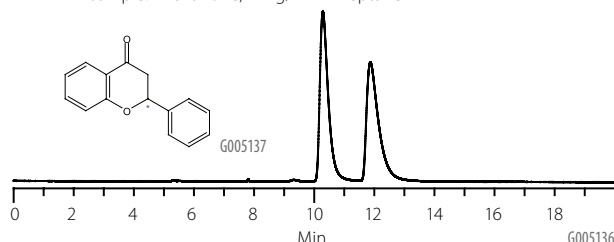


Figure 9. Oxazepam on Astec CYCLOBOND I 2000 DNP

column: CYCLOBOND I 2000 DNP, 25 cm x 4.6 mm I.D.,
5 μ m particles (25024AST)
mobile phase A: acetonitrile
mobile phase B: 20 mM ammonium phosphate, pH 2.9
mobile phase ratio: 20:80 (A:B)
flow rate: 1 mL/min.
temp.: 5 $^{\circ}$ C
det.: UV, 254 nm
injection: 3 μ L
sample: oxazepam, 1 mg/mL in acetonitrile:water (50:50)

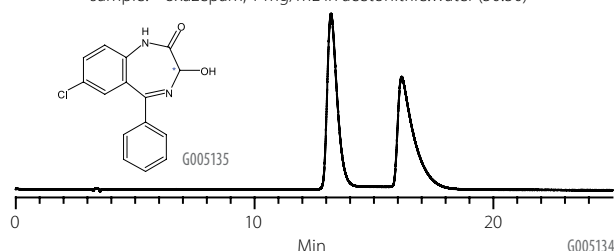
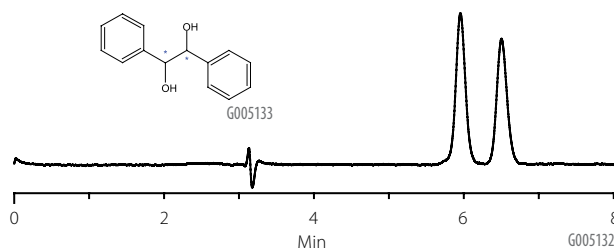


Figure 10. Hydrobenzoin on Astec CYCLOBOND I 2000 RSP

column: CYCLOBOND I 2000 RSP, 25 cm x 4.6 mm I.D.,
5 μ m particles (20324AST)
mobile phase A: acetonitrile
mobile phase B: 10 mM ammonium acetate, pH 4.0
mobile phase ratio: 25:75 (A:B)
flow rate: 1 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 254 nm
injection: 5 μ L
sample: hydrobenzoin, 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 π -basic phase similar in character to the naphthylethyl carbamate phases. The selectivity is greater for the CYCLOBOND I 2000 DMP 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 DNP*

β -Cyclodextrin, 2,6-Dinitro-4-trifluoromethyl phenyl ether derivative

This unique derivative has dinitrophenyl functionality bonded through an ether linkage to the β -CD. In this arrangement, a π -electron sharing system is established with analytes having π -systems in the stereogenic environment (e.g., aromatic rings, carbonyls). The π -acidity of this group is further enhanced with the introduction of the trifluoromethyl group in the aromatic ring. Use of the ether linkage to anchor this π -acidic dinitrophenyl ring results in a very stable system even under strong reversed-phase conditions. In a number of cases, selectivity was demonstrated only on CYCLOBOND I 2000 DNP; examples are ketorolac, oxazepam and compounds with multiple ring systems. While CYCLOBOND I 2000 DNP demonstrates selectivity in all three mobile phase conditions, reversed-phase conditions yield the greatest number of separations and highest selectivity. It has also been observed that buffers can have a dramatic affect on selectivity, especially when employing ammonium phosphate.

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.

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

In the design of this new chemistry, it was an objective to create a very stable and reproducible phase with shorter retention times, while maintaining or improving selectivity compared with CYCLOBOND I 2000 RSP. After an extensive evaluation of this new chemistry, that goal was attained, as well as a dramatic improvement in a number of separations. CYCLOBOND I 2000 HP-RSP 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, 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 has the affect 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 is γ -cyclodextrin bonded to silica. An excellent chiral selector for multi-ring structures, it is useful for isomeric compounds based on anthracene, chrysene and pyrene-type ring structures. CYCLOBOND II offers good selectivity and stability, and is applicable to the polar organic and reversed-phase modes. Applications include steroids, porphyrins, and Fmoc amino acids.

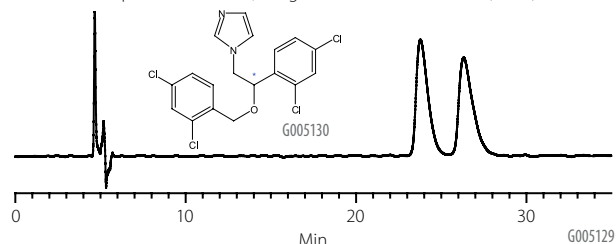
Astec CYCLOBOND II AC *γ -Cyclodextrin, acetylated*

CYCLOBOND II AC columns utilize γ -cyclodextrin with the hydroxyl groups in the 2 and 3 position acetylated. As a result, the mouth of the cavity has a hydrogen acceptor site suitable to interact with a hydrogen donor, such as an amine attached to at least two fused rings or larger. An example would be 1- or 2- substituted naphthylethylamine. Applications include steroids and sterols, depending on where the hydroxyl groups are positioned.

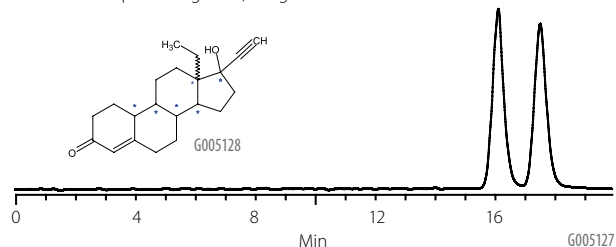
* Phases included in Method Development Kit (20005AST).

Figure 11. Miconazole on Astec CYCLOBOND I 2000 HP-RSP

column: CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D.,
5 μ m particles (24024AST)
mobile phase A: acetonitrile
mobile phase B: 20 mM ammonium acetate, pH 4.0
mobile phase ratio: 25:75 (A:B)
flow rate: 0.6 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 230 nm
injection: 5 μ L
sample: miconazole, 1 mg/mL in acetonitrile:water (50:50)

**Figure 12. Norgestrel on Astec CYCLOBOND II**

column: CYCLOBOND II, 25 cm x 4.6 mm I.D.,
5 μ m particles (41020AST)
mobile phase A: water
mobile phase B: acetonitrile
mobile phase ratio: 70:30 (A:B)
flow rate: 0.8 mL/min.
temp.: 22 $^{\circ}$ C
det.: UV, 254 nm
injection: 1 μ L
sample: norgestrel, 1 mg/mL in methanol




Method Development on CYCLOBOND Columns

Astec CYCLOBOND columns should be part of any chiral HPLC column screening protocols.


With that in mind, we have developed column screening and optimization protocols and published them in a convenient wall chart format (T409107).

To request a copy, please contact your local Sigma-Aldrich/Supelco office or visit our website:

sigma-aldrich.com/chiral



Chiral HPLC Method Development Screen on Astec CHIROBIOTIC® & CYCLOBOND™



Introduction

These screening protocols are used by our Chiral Applications Laboratory personnel to provide a rapid determination of the most suitable CHIROBIOTIC® or CYCLOBOND column and mobile phase combination for an enantiomer separation.

CHIROPOTIC Screening Protocol

Mobile Phase System

Polar solvent (PMS)	CH ₂ Cl ₂ or CH ₂ Cl ₂ /20 mM ammonium acetate, pH 5.0 (50:50)
Reversed phase (RP)	CH ₂ Cl ₂ /20 mM ammonium acetate, pH 5.0 (50:50)
Polar organic (PO)	Ethanol
Normal phase (NP)	Ethanol/Hexane (50:50)

Conditions for 25 cm x 4.6 mm I.D. column: Flow rate: 1.0 mL/min, Sample: 2.0 µL of 1.2 mg/mL in CH₂Cl₂ or CH₂Cl₂/Hexane

CYCLOBOND Screening Protocol

Mobile Phase System

Polar solvent (PMS)	CH ₂ Cl ₂ /20 mM ammonium acetate, pH 5.0 (50:50)
Reversed phase (RP)	CH ₂ Cl ₂ /20 mM ammonium acetate, pH 5.0 (50:50)
Polar organic (PO)	CH ₂ Cl ₂ /CH ₃ OH/acetic acid/TEA (50:5:5:10:10)
Normal phase (NP)	Ethanol/Hexane (50:50) (EAP and DAP only)

Conditions for 25 cm x 4.6 mm I.D. column: Flow rate: 1.0 mL/min, Sample: 2.0 µL of 1.2 mg/mL in CH₂Cl₂ or CH₂Cl₂/Hexane

Optimization

Mobile Phase System

Test and base ratios from 1:4 to 4:1 to alter retention and selectivity.

Typical acid and base concentrations are 0.01 to 1%.

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.

Ammonium can be added up to 50%.

Change % and type of organic modifier.

Adjust pH, buffer type, ionic strength.

Use other polar organic solvents or blends (e.g. combinations of CH₂Cl₂, CH₃OH, MTBE).

(CYCLOBOND) Test and base ratios from 1:4 to 4:1 to alter retention and selectivity.

Typical acid and base concentrations are 0.01 to 1%.

Increase % of polar modifier.

Change both solvents (e.g. 8% for ethanol, test any organic solvent).

LC-MS Optimization

CHIROPOTIC

Use volatile salts in PMS (e.g. ammonium acetate, ammonium formate, ammonium trifluoroacetate).

CYCLOBOND

Replace TEA with ammonium hydroxide in PMS, lower concentration by 50-75%.

Both columns

Use ammonium acetate or ammonium formate in NP.

Column Reconditioning & Storage

CHIROPOTIC Columns

Conditioning: Flush columns with 10 column volumes of acetonitrile (50:50) to wet the resin, then flush with 10 column volumes of water. Then flush with 10 column volumes of 10% acetonitrile in water. Then flush with 10 column volumes of 10% acetonitrile in water.

CYCLOBOND Columns

Conditioning: Flush columns with 10 column volumes of acetonitrile (50:50) to wet the resin, then flush with 10 column volumes of water. Then flush with 10 column volumes of 10% acetonitrile in water. Then flush with 10 column volumes of 10% acetonitrile in water.

Operating Parameters & Compatibility

CHIROPOTIC and CYCLOBOND columns are chemically bonded and compatible with all conventional HPLC solvents and buffers. The only critical operating parameter to avoid is pH outside the recommended range.

pH Range	3 to 7
Temperature	5 to 35 °C
Pressure	< 1500 psi (100 bar)
Flow Rate	0.1 to 1.0 mL/min (250 µL/min)
Column Size	25 cm x 4.6 mm I.D.

Flow Rate: Column flow rate for 25 cm x 4.6 mm I.D. column is 1.0 mL/min (250 µL/min). Test optimum for your separation.

Chiral Services

Chiral column screening, method optimization, and small-scale enantiomer purifications are available from Supelco Analytical. For more information on our chiral services, please contact our web site, sigma-aldrich.com/chiral or contact our Technical Service Department.

US & Canada: Phone: 800-359-3041 / 800-359-3041
Fax: 800-359-3041 / 800-359-3041
Email: techservice@supelco.com

sigma-aldrich.com/chiral

SIGMA-ALDRICH®

Supelco Chiral Services

Our Chiral Services Laboratory is distinct in offering HPLC column screening in normal phase and LC-MS-compatible reversed-phase, polar ionic and polar organic separation modes, using Astec Cellulose, CHIROBIOTIC, CYCLOBOND, P-CAP and other CSPs and modes as dictated by sample solubility and specific customer requirements. Consult Supelco to obtain a quotation for our expert services for chiral column screening (HPLC and GC), method optimization, and isolation of up to 10 grams of purified enantiomer. Purifications above 10 grams to production scale are available through our SAFC partners worldwide. The complete listing of our chiral HPLC and GC columns and chiral services can be found at sigma-aldrich.com/chiral, our corporate chiral web portal. Here you can view our other products for chiral chemistry, like chiral catalysts, building blocks, mobile phase additives, derivatization reagents, and more.



P001331



Astec CYCLOBOND Columns

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

Cat. No.	Particle Size (µm)	Length (cm)	I.D. (mm)
Astec CYCLOBOND I 2000			
20019AST	5	15	2.1
20020AST	5	25	2.1
20023AST	5	15	4.6
20024AST	5	25	4.6
20034AST	5	25	10
20044AST	5	25	21.2
Guards			
21010AST	5	2	1
21100AST*	5	2	4
Astec CYCLOBOND I 2000 RSP			
20319AST	5	15	2.1
20320AST	5	25	2.1
20323AST	5	15	4.6
20324AST	5	25	4.6
20334AST	5	25	10
20344AST	5	25	21.2
Guards			
21013AST	5	2	1
21103AST*	5	2	4
Astec CYCLOBOND I 2000 DMP			
20719AST	5	15	2.1
20720AST	5	25	2.1
20723AST	5	15	4.6
20724AST	5	25	4.6
20734AST	5	25	10
20744AST	5	25	21.2
Guards			
21017AST	5	2	1
21107AST*	5	2	4
Astec CYCLOBOND I 2000 SP			
20219AST	5	15	2.1
20220AST	5	25	2.1
20223AST	5	15	4.6
20224AST	5	25	4.6
20234AST	5	25	10
20244AST	5	25	21.2
Guards			
21012AST	5	2	1
21102AST*	5	2	4

Cat. No.	Particle Size (µm)	Length (cm)	I.D. (mm)
Astec CYCLOBOND I 2000 AC			
20119AST	5	15	2.1
20120AST	5	25	2.1
20123AST	5	15	4.6
20124AST	5	25	4.6
20134AST	5	25	10
20144AST	5	25	21.2
Guards			
21011AST	5	2	1
21101AST*	5	2	4
Astec CYCLOBOND I 2000 HP-RSP			
24019AST	5	15	2.1
24020AST	5	25	2.1
24023AST	5	15	4.6
24024AST	5	25	4.6
24034AST	5	25	10
24044AST	5	25	21.2
Guards			
24101AST	5	2	1
24100AST*	5	2	4
Astec CYCLOBOND I 2000 DNP			
25019AST	5	15	2.1
25020AST	5	25	2.1
25023AST	5	15	4.6
25024AST	5	25	4.6
25034AST	5	25	10
25044AST	5	25	21.2
Guards			
25101AST	5	2	1
25100AST*	5	2	4
Astec CYCLOBOND I 2000 DM			
20919AST	5	15	2.1
20920AST	5	25	2.1
20923AST	5	15	4.6
20924AST	5	25	4.6
20934AST	5	25	10
20944AST	5	25	21.2
Guards			
21019AST	5	2	1
21109AST*	5	2	4



Guard Column Holders



P001316

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

Cat. No.	Description
21150AST	Guard Column Holder

Cat. No.	Particle Size (μm)	Length (cm)	I.D. (mm)
Astec CYCLOBOND II			
46019AST	5	15	2.1
41021AST	5	25	2.1
46023AST	5	15	4.6
41020AST	5	25	4.6
40025AST	5	25	10
40028AST	5	25	21.2
Guards			
41001AST	5	2	1
42120AST*	5	2	4
Astec CYCLOBOND IIAC			
47019AST	5	15	2.1
41024AST	5	25	2.1
47023AST	5	15	4.6
41022AST	5	25	4.6
40420AST	5	25	10
40422AST	5	25	21.2
Guards			
41002AST	5	2	1
42121AST*	5	2	4

* Requires guard column holder (21150AST)..

Astec CYCLOBOND Method Development Kit

The kit contains one 25 cm x 4.6 mm I.D. column of each of our four most popular Astec CYCLOBOND phases: CYCLOBOND I 2000, CYCLOBOND I 2000 HP-RSP, CYCLOBOND I 2000 DMP, and CYCLOBOND I 2000 DNP. Because the price of the kit is below that of the columns sold separately, it is an economical means to augment your chiral column library.

Cat. No.	Description
20005AST	Astec CYCLOBOND Method Development Kit

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