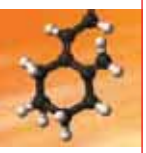

Innovations in Chiral Chromatography

Overview of Modern Chiral Stationary Phases

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Through our own Astec line and partnerships with other innovative companies, Supelco offers the widest range of chiral stationary phase (CSP) classes for HPLC, GC, and SFC. They form part of Sigma-Aldrich's "universal" chiral offering that also includes reagents, chiral catalysts, cocrystallization, services, and more. This article will describe the major CSPs for HPLC and SFC in use today. Subsequent articles in this series will focus on putting them to practical use.

One of the most specialized areas of chromatography deals with the separation of enantiomers. Since the discovery of optical activity in the early 19th century, materials and techniques have evolved to separate and purify enantiomers. Chromatography has become an important tool for this purpose, and analysts today have many CSPs from which to choose. The dynamic abundance of CSPs is necessary; each enantiomer separation is unique and requires specific differentiating interactions.

Common Features of Modern CSPs

The chiral selectors of today's successful CSPs are based on or mimic complex biomolecules, like proteins, peptides, and carbohydrates. This is no coincidence. It is because biomolecules can distinguish enantiomers that biological systems recognize chirality. Biomolecules are also rich in the number and diversity of chiral recognition sites, both structural and chemical. This helps both enantioselectivity and capacity.

- **Structural:** Pockets or other 3-dimensional regions distinguish molecular shape
- **Chemical:** Functional groups provide specific and differentiating interactions

Modern CSPs generally rely on spherical, porous silica gel as the underlying support particle. Silica has advantages of efficiency, stability, and ease of modification over synthetic polymer particles. So, although there are exceptions, CSPs for HPLC and SFC typically are silica particles bonded or coated with native, modified, or mimetic biomolecules.

Polysaccharides (cellulose, amylose)

The most popular class of CSPs for HPLC and SFC, the polysaccharides amylose and cellulose are naturally-occurring, optically-active, linear (cellulose) and helical (amylose) polymers comprising hundreds to thousands of D-(+)-glucose units joined by α(1→4) glycosidic (amylose) bonds or ß(1→4) glycosidic (cellulose) bonds. The long polysaccharide chains form rope-like bundles held together via multiple hydrogen bonds between proximate hydroxyl groups. Derivatized cellulose- and amylose-based CSPs 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. An example chromatogram is shown in **Figure 1.** (1, 2)

Figure 1. Demonstration of Cellulose Used as a CSP (Etodolac Enantiomers)

column: Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase: 10:90:0.1, IPA:heptane:TFA
flow rate: 0.5 mL/min.
temp.: 25 °C
det.: UV, 254 nm

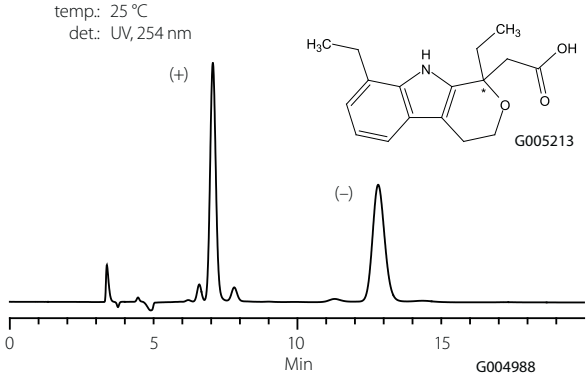
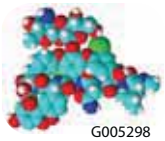


Table 1. Selection of Chiral HPLC and SFC phases from Sigma-Aldrich

Class	Chiral Selectors (phases)	Product Line
Polysaccharide	tris-(3,5-dimethylphenyl) carbamoyl cellulose	Astec Cellulose DMP, Kromasil® CelluCoat
	tris-(3,5-dimethylphenyl)carbamoyl amylose	Kromasil AmyCoat
Macrocyclic glycopeptide	teicoplanin, teicoplanin aglycone, vancomycin, ristocetin A	Astec CHIROBIOTIC®
Cyclodextrin	ß- and γ-cyclodextrins, native and derivatized	Astec CYCLOBOND®
Protein	α1-acid glycoprotein, cellobiohydrolase, albumin (human serum)	Chiral-AGP, Chiral-CBH, Chiral-HSA
Chiral synthetic polymer	poly(trans-1,2-cyclohexanediyl-bis-acrylamide)	Astec P-CAP™
	poly(diphenylethylenediamine-bis-acryloyl)	Astec P-CAP-DP
	O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-L-tartar diamide	Kromasil Chiral DMB
	O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide	Kromasil Chiral TBB
Chiral ligand exchange	chiral bidentate ligand	Astec CLC-L, Astec CLC-D
Cyclofructan	derivatized cyclofructan 6	LARIHC™

Macrocyclic Glycopeptides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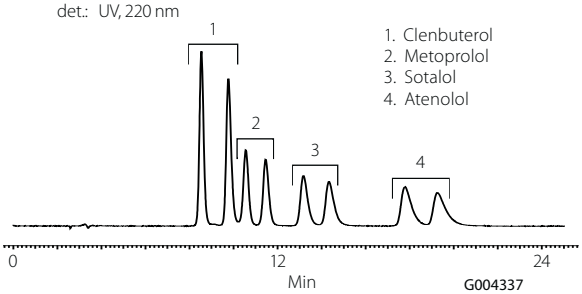
This successful class of CSPs uses naturally-occurring macrocyclic glycopeptides as the chiral selector. They offer five different types of molecular interactions: ionic, H-bond, π-π, dipole, and hydrophobic, and multiple inclusion sites that influence selectivity based on the molecular shape of the analyte. Ionic interactions are unique to these CSPs, and are responsible for their success with polar and ionizable analytes, and their utility in reversed-phase and LC-MS mobile phases. An example chromatogram is shown in **Figure 2.** (3)

Figure 2. Macrocyclic Glycopeptide Used as a CSP (Enantiomers of ß-Blockers)

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



Cyclodextrins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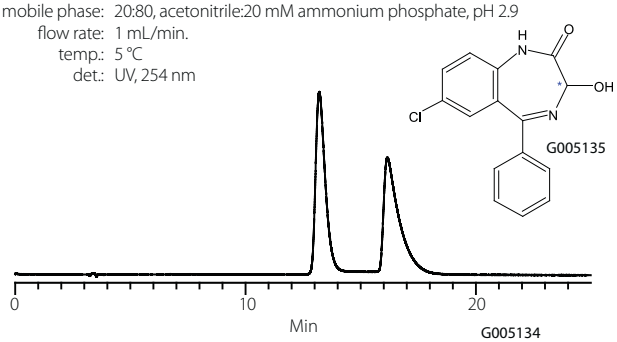
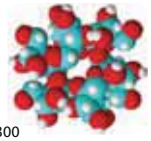
Cyclodextrins (CDs) comprise D-(+)-glucose residues bonded through α(1→4) glycosidic linkages. The chair configuration of glucose makes the toroid bucket narrower at one end. Derivatization of the 2- and 3-position hydroxyl groups affects selectivity. Enantioseparations occur on the inside (inclusion complexing) and outside surfaces (surface interactions). The most important consideration for retention and chiral recognition is proper fit of the analyte into the CD cavity. This fit is a function of both molecular size and shape of the analyte relative to the cavity. An example chromatogram is shown in **Figure 3.** (4)

Figure 3. ß-Cyclodextrin Used as a CSP (Oxazepam Enantiomers)

column: Astec CYCLOBOND I 2000 DNP, 25 cm x 4.6 mm I.D., 5 µm particles (25024AST)
mobile phase: 20:80, acetonitrile:20 mM ammonium phosphate, pH 2.9
flow rate: 1 mL/min.
temp.: 5 °C
det.: UV, 254 nm



Cyclofructans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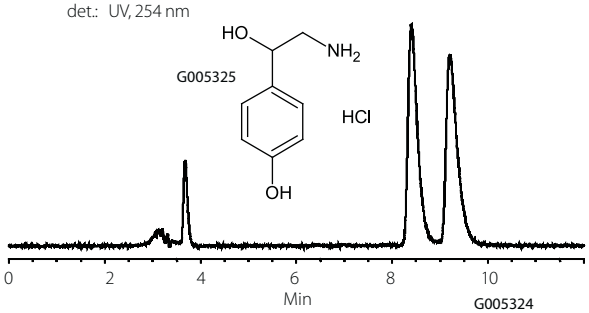
Cyclofructans are the newest class of CSPs. They comprise six or more ß(2→1) linked D-fructofuranose units. Although structurally similar to cyclodextrins, they have very different selectivity. The propyl derivative is particularly adept at separating chiral primary amines **(Figure 4)**. (5)

Figure 4. Cyclofructan-6 Used as a CSP (Octopamine Enantiomers)

column: LARIHC™ CF6-P, 25 cm x 4.6 mm I.D., 5 µm particles (AZYP Part No. L1001, available from Supelco/Sigma-Aldrich as a custom item)
mobile phase: 70:30:0.3.0.2, methanol:acetonitrile:acetic acid:triethylamine
flow rate: 1 mL/min.
temp.: 20 °C
det.: UV, 254 nm



Proteins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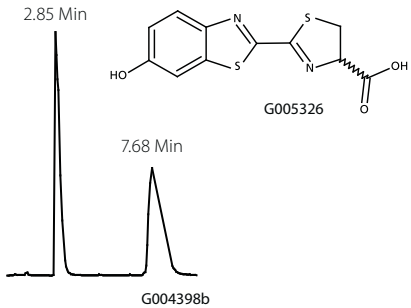
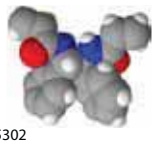
Proteins contain a large number of chiral centers and many other sites that contribute to the general retention process. Three proteins that have been particularly successful as CSPs are α1-acid glycoprotein (AGP, shown in **Figure 5**), cellobiohydrolase (CBH), and human serum albumin (HSA). (6)

Figure 5. Protein (AGP) Used as a CSP (Luciferin Enantiomers)

column: Chiral-AGP, 10 cm x 4 mm I.D., 5 µm particles (58150AST)
mobile phase: 10 mM sodium phosphate, pH 6.0
flow rate: 0.9 mL/min.
temp.: 25 °C
det.: UV, 225 nm



Chiral Synthetic Polymers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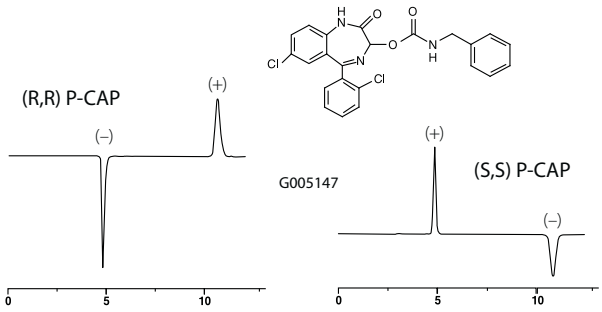
Synthetic CSPs have a defined structure and controlled degree of polymerization, and mitigate certain drawbacks associated with natural compounds. Most comprise a thin, ordered layer of chiral polymer covalently bonded to the silica surface. Because they are synthetic, they can be identically manufactured in both R,R and S,S forms, providing a predictable reversal of elution order. An example is shown in **Figure 6.** (7-9)

Figure 6. Chiral Synthetic Polymer Used as a CSP (Furoin Enantiomers)

columns: 25 cm x 4.6 mm I.D., 5 µm
mobile phase: 95:5, methylene chloride:methanol
flow rate: 1 mL/min.



Chiral Ligand Exchange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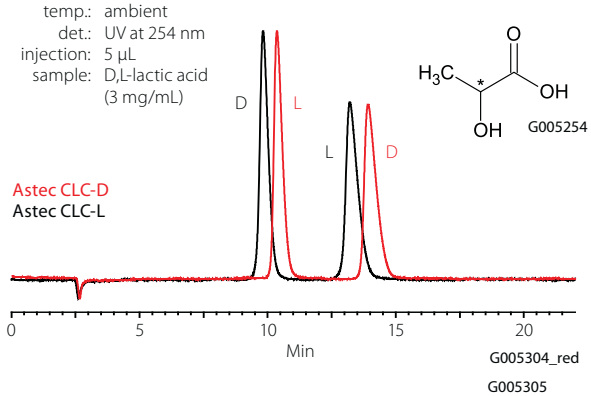
Copper ions in the mobile phase coordinate with the chiral selector on the stationary phase (a small, chiral bidentate ligand) and carboxylic acid functional groups on the analytes to form transient diastereomeric complexes in solution. Analytes include alpha-hydroxy acids, like lactic, malic, tartaric, and mandelic acids, amino acids, other amines and bifunctional racemates, like amino alcohols. The technique also gives analytes a strong 254 nm signal. Two versions (D and L, **Figure 7**) provide elution order reversal. (10)

Figure 7. Chiral Ligand Exchange Chromatogram (Lactic Acid Enantiomers)

columns: Astec CLC-D (53023AST) and Astec CLC-L (53123AST), both 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 5 mM $CuSO_4$
flow rate: 1.0 mL/min.
temp.: ambient
det.: UV at 254 nm
injection: 5 µL
sample: D,L-lactic acid (3 mg/mL)



Conclusion

Irrespective of the success of the CSPs discussed in this article, there is plenty of room in the field for other types. Continue to look to Supelco for innovative, practical solutions for chiral separations.

Visit our chiral web portal *sigma-aldrich.com/chiral* to learn more about Sigma-Aldrich's wide range of products and services for chiral chemistry and separations.

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Related Information

For further reading:

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