# Astec Cellulose DMP



Efficient, Rugged, and Economical Columns for Chiral HPLC & SFC



Efficient, rugged, reproducible, and scalable

Low backpressure

Essential component of chiral column screening

**Economically priced** 



# Astec Cellulose DMP

Astec Cellulose DMP 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. It 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 is complementary to the other Astec CSPs, including CHIROBIOTIC®, CYCLOBOND™, and the P-CAP™ 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

### What is Cellulose?

The polysaccharide cellulose is a naturally occurring, optically active, linear polymer comprising hundreds to thousands of D-(+)-glucose units joined by  $\beta$ 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 lead 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.

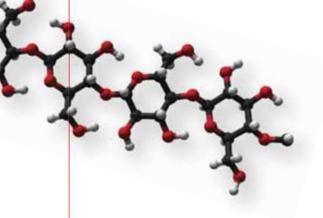


Figure 1. DMPC-Cellulose Structure

$$R = \bigcup_{OR}^{OR} \bigcup_{OR}^{OR}$$

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

## How do Cellulose-Based CSPs Separate Enantiomers?

Derivatized cellulose-based CSPs, like Astec Cellulose DMP, 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 (Figure 1) separates a wide range of enantiomers; the phenyl ring and carbamate groups provide  $\pi$ – $\pi$  and hydrogen bonding interactions, respectively, with predisposed analytes.

# What Makes Astec Cellulose DMP Unique?

Astec Cellulose DMP is unique in offering classic DMPC-cellulose chiral selectivity, but at approximately one-half the cost of most DMPC-cellulose columns. It 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 IPA as polar modifiers. The performance of Astec Cellulose DMP in terms of selectivity and compatibility under normal phase conditions meets or exceeds competitive phases of similar composition. Figures 2 - 4 show the resolution of three racemates on Astec Cellulose DMPC and two higherpriced competitive phases.

# Comparable to Other DMPC-Cellulose Columns

For the compounds we have tested, Astec Cellulose DMP columns provide similar retention and selectivity, but lower backpressure and higher efficiency compared to competitive columns. Astec Cellulose DMP 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 your chiral column HPLC and SFC screening protocols. It also should be investigated as a possible replacement for these columns in established methods. The data in **Table 1** compares resolution, pressure, and selectivity on Astec Cellulose DMP and the leading competitive column. Comparison also appears in **Figures 2 - 4** against two competitive columns.

Table 1. Comparison of Astec Cellulose DMP vs. Leading Competitor for Sample Set of Chiral Compounds

	AA - I-ii -	Astec Cellulose DMP			Competitor D				
Compound	Mobile Phase*	Pressure (bar)	Selectivity	N (Peak 1)	Resolution	Pressure (Bar)	Selectivity	N (Peak 1)	Resolution
Alprenolol	А	16	3.0	9,528	12.8	19	1.4	5,963	3.5
Atropine	А	16	1.4	5,768	4.9	19	1.7	3,666	6.1
Benzoin	В	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	В	16	2.6	6,323	10.2	19	2.8	5,568	10.1
Hydroxyzine	А	16	1.2	5,477	1.9	19	1.2	4,173	2.1
Ketamine	А	16	1.2	9,506	2.4	19	1.2	8,172	2.3
Metoprolol	А	16	2.3	7,208	7.0	19	2.6	5,343	6.0
Mianserin	А	16	1.2	6,936	1.9	19	1.2	6,078	1.6
Proglumide	В	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	А	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 particles, flow rate: 0.5 mL/min., temp.: 25 °C (Note: Separations not optimized on either column)

\* Mobile Phase A: 10:90:0.1, IPA:heptane:DEA Mobile Phase B: 10:90:0.1, IPA:heptane:TFA

Mobile Phase C: 0.1% w/v ammonium formate in methanol

Mobile Phase D: 10:90, IPA:heptane

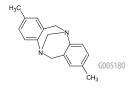
#### Figure 2. Tröger's Base - Competitive Comparison

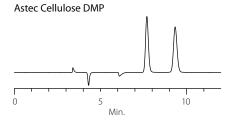
columns: 15 cm x 4.6 mm l.D., 5 µm particles

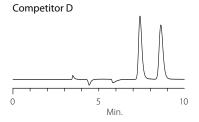
mobile phase: 10:90:0.1, IPA:heptane:DEA

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 230 nm inj.: 2 μL

sample: Tröger's Base (2 mg/mL)







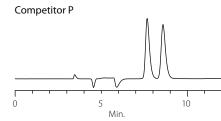
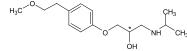
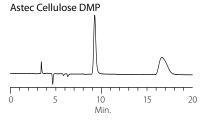


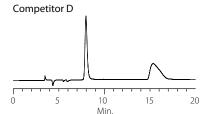


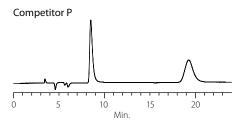
Figure 3. Metoprolol - Competitive Comparison

Conditions same as Figure 2 except: sample: metoprolol







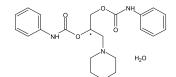


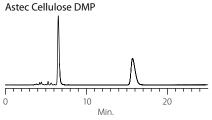
#### Figure 4. Diperodon - Competitive Comparison

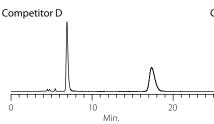
Conditions same as Figure 2 except:

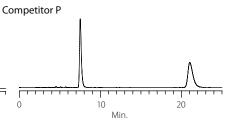
mobile phase: 0.1% ammonium formate in methanol

sample: diperodon









## Normal Phase Applications

Figure 5. Alprenolol

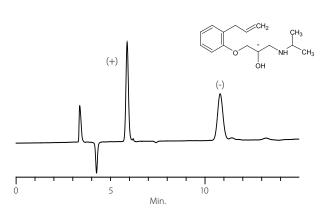
Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu$ m particles

(51098AST)

10:90:0.1, IPA:heptane:DEA mobile phase:

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 254 nm inj.: 2 μL

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



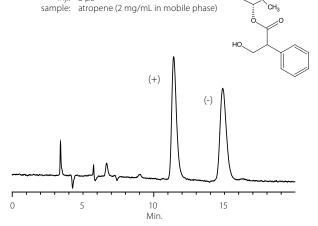
ORDER: 800-247-6628 (US only) / 814-359-3441

#### Figure 6. Atropine

Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu$ m particles (51098AST)

mobile phase: 10:90:0.1, IPA:heptane:DEA

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 254 nm inj.: 2 μL



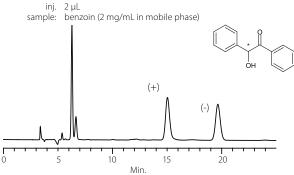
#### Figure 7. Benzoin

Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu$ m particles column:

(51098AST)

mobile phase: 10:90:0.1, IPA:heptane:TFA

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 254 nm



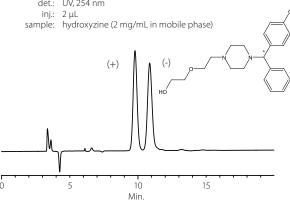
#### Figure 8. Hydroxyzine

column: Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu$ m particles

(51098AST)

10:90:0.1, IPA:heptane:DEA

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 254 nm inj.: 2 µL



#### Figure 9. Proglumide

column: Astec Cellulose DMP, 15 cm x 4.6 mm l.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 inj.: 2 μL sample: proglumide (2 mg/mL in mobile phase) (+)(-)

#### Figure 10. Etodolac

Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu m$  particles column:

(51098AST)

mobile phase: 10:90:0.1, IPA:heptane:TFA

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 254 nm inj.: 2 μL

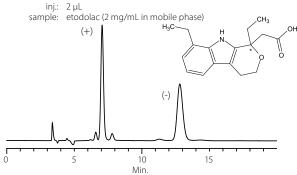


Figure 11. Ketamine

column: Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu$ m particles

(51098AST)

mobile phase: 10:90:0.1, IPA:heptane:DEA

flow rate: 0.5 mL/min. 25 °C temp.: inj.: 2 μL

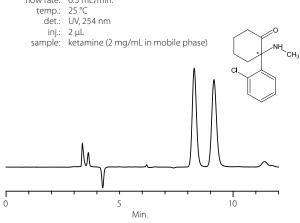


Figure 12. Warfarin

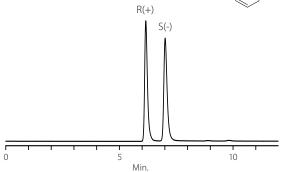
column: Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5 µm particles

(51098AST)

mobile phase: 100:0.2:0.1, CH<sub>3</sub>OH:acetic acid:TEA

flow rate: 0.5 mL/min. temp.: 25 °C det.: UV, 278 nm

inj.: 2 µL sample: warfarin (2 mg/mL in mobile phase)





10

Min.

15

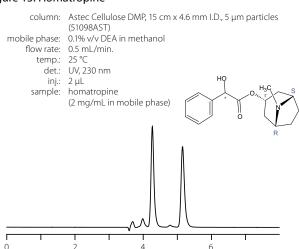


#### Polar Organic Mode Chiral Separations

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 operates in POM to permit choice of mobile phase based on analyte solubility. A few of the racemates resolved on Astec Cellulose DMP with different POM mobile phases appear in Table 2 and Figures 13-15.

Not only does Astec Cellulose DMP 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. Figure 16 demonstrates the same Astec Cellulose DMP column used alternately in normal phase and POM mobile phases. Performance values appear in the figure.

Figure 13. Homatropine



Min

Figure 14. Indapamide

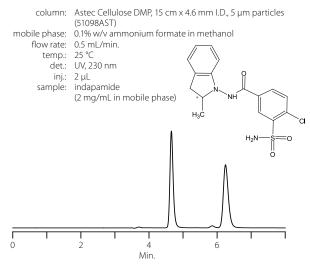
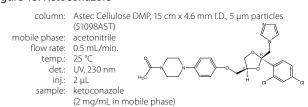


Figure 15. Ketoconazole



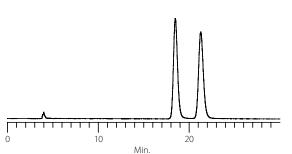


Table 2. Performance of Astec Cellulose DMP in Polar Organic Mode (POM)

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 <sub>R</sub> Peak 1 (min.)	Selectivity	t <sub>R</sub> Peak 1 (min.)	Selectivity	t <sub>R</sub> 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 l.D., 5 µm particles (51098AST), flow rate: 0.5 mL/min., temp.: 25 °C \*Warfarin mobile phase: 100:0.2:0.1, methanol:acetic acid:TEA

# Figure 16. Stable Performance After Repeated NP-POM Cycles

columns: Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5  $\mu$ m particles

(51098AST)

mobile phase

(normal phase): 10:90, IPA:heptane

mobile phase

(POM): 0.1% w/v ammonium formate in methanol

flow rate: 0.5 mL/min.

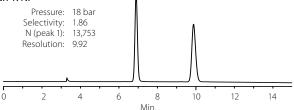
temp.: 25 ℃

sample: TSO (normal phase) or mianserin (POM)

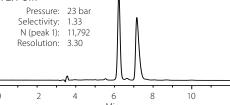
det.: UV, 254 nm

inj.: 2 μL

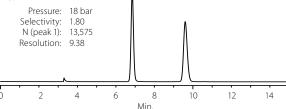
#### Run 1: NP



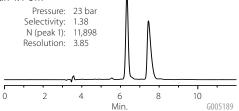
#### Run 2: POM



#### Run 3: NP



#### Run 4: POM



#### Analyte Structure:

trans-Stilbene Oxide





### SFC Chiral Separations

SFC (supercritical fluid chromatography) is gaining in popularity, especially for chiral separations, due to its speed advantages over HPLC. The  $\mathrm{CO}_2$  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 works well in SFC mode, providing rapid separations with excellent selectivity. For example, **Figure 17** shows the SFC separation of a mixture of six diastereomers of a single compound in less than four minutes on Astec Cellulose DMP. As a testament to its utility as a screening tool, the durability of Astec Cellulose DMP permits rapid (ballistic) gradients of methanol, ethanol or IPA in  $\mathrm{CO}_2$  with long column lifetime and low backpressure, but without significant column bleed (**Figure 18**).

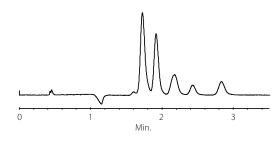
#### Figure 17. Rapid SFC Separations

column: Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5 µm particles

(51098AST)
mobile phase: 10% ethanol in CO<sub>2</sub>
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)



# Figure 18. Stability and Resolution under Ballistic SFC Gradients

Conditions same as Figure 17 except:

gradient: 5-65% methanol in CO<sub>2</sub>; hold 1 min. flow rate: 4 ml /min.

SFC data for Figures 17 and 18 kindly provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.





# **Ideal for Preparative Applications**

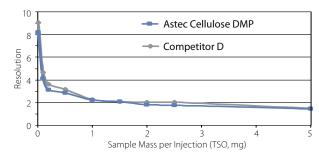
A characteristic of the polysaccharide-based CSPs that has contributed to their popularity is their utility for preparative applications. Although there are many factors to consider in a preparative scale up, the particle contribution comes primarily from the amount of available stationary phase and to what degree it resolves the enantiomers. When we designed the Astec Cellulose DMP, we sought 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 is comparable to Competitor D (Figure 19). 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 Figure 20 for the anti-Alzheimer's drug BAY 73-6691.

#### Figure 19. Loading Capacity

columns: 15 cm x 4.6 mm l.D., 5 μm particles mobile phase: 10:90, IPA:hexane

flow rate: 1 mL/min. temp.: 28° C det.: UV, 210 nm inj.: 100 μL

sample: trans-stilbene oxide (TSO), 0.05 – 50 mg/mL



#### Figure 20. Scale-Up Example

columns: Astec Cellulose DMP, 5 µm particles

mobile phase: 80:10:10, heptane:methyl tert-butyl ether (MTBE):ethanol

temp.: 25 °C det.: UV, 230 nm

sample: BAY 73-6691 in mobile phase

#### Analytical:

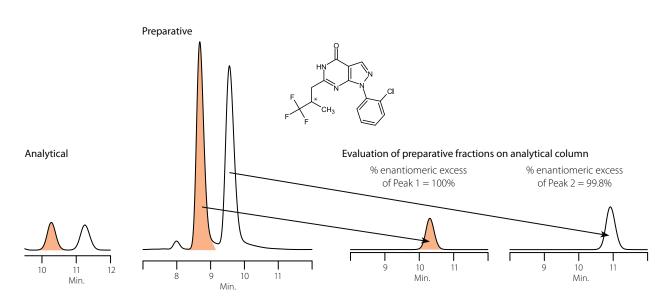
dimensions: 15 cm x 4.6 mm I.D. (51098AST)

flow rate: 0.5 mL/min. inj.: 10  $\mu$ L (2 mg/mL)

Preparative:

dimensions: 25 cm x 21.2 mm I.D. (51103AST)

flow rate: 13 mL/min. inj.: 5000  $\mu$ L (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 is a coated phase, the DMPC-cellulose is held securely onto the silica surface. Figure 21 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. Also note the discussion about operation in polar organic mode in Table 2 and Figure 16. The Astec Cellulose DMP 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.)

#### Figure 21. Stability Demonstration

column: Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles

(51098AST)
mobile phase: 10:90, IPA:heptane
flow rate: 1 mL/min.
temp.: 28 °C
det.: UV, 210 nm

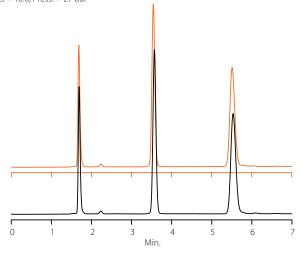
inj.: 2 μL sample: TSO, 1 mg/mL

#### 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. We have designed Astec Cellulose DMP to have the reproducibility you require for method validation. The example in **Figure 22** shows columns from three different production lots of Astec Cellulose DMP in both normal phase and polar organic modes.

#### Figure 22. Reproducibility Demonstration

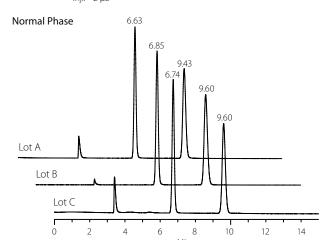
column: Astec Cellulose DMP, 15 cm x 4.6 mm l.D., 5 μm particles (51098AST)
mobile phase
(normal phase): 10:90, IPA:heptane
mobile phase
(POM): 0.1% w/v ammonium formate in methanol
flow rate: 0.5 mL/min.

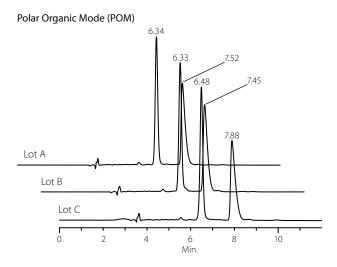
sample: TSO (normal phase) or mianserin (POM)

det.: UV, 230 nm inj.: 2 μL

25 °C

temp.:









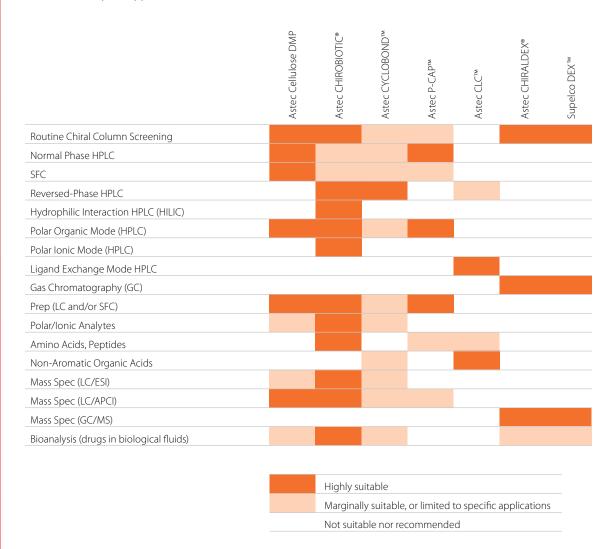
# Complementary to the Other Astec CSPs

Cellulose DMP is the latest addition to the Astec line of highquality chiral stationary phases manufactured by Sigma-Aldrich. Other Astec HPLC CSPs include CHIROBIOTIC, CYCLOBOND, P-CAP, P-CAP-DP, CLC-L, and CLC-D. Astec CHIRALDEX and Supelco DEX 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 means:

- It is likely that at least one Astec CSP will give the necessary selectivity. Incorporating Astec Cellulose DMP, CHIROBIOTIC and CYCLOBOND in your 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 Table 3.

Table 3. Techniques, Applications, and Fields of Use for Astec Chiral Phases



#### Ordering Information

#### **Astec Cellulose DMP Columns**

Particle Size (μm)	Length (cm)	I.D. (mm)	Cat. No.
5	15	2.1	51100AST
5	25	2.1	51101AST
5	10	4.6	51097AST
5	15	4.6	51098AST
5	25	4.6	51099AST
5	25	10	51102AST
5	25	21.2	51103AST

#### **Test Mix**

Description	Package Size	Cat. No.			
Test mix for polysaccharide-based	1 mL	40119-U			
chiral HPLC columns, contains trans-stilbene					
oxide (TSO) and 1,3,5-tri-tert-butylbenzene ( $V_0$ marker),					
each 30 μg/mL in hexane					

#### Guards

Description	Length (cm)	I.D. (mm)	Cat. No.
Guard column kit for 2.1 mm I.D. columns. Contains holder, one cartridge, 2" x 1/16" tubing, two nuts, and two ferrules	2	2.1	51105AST
Replacement guard cartridges for 2.1 mm I.D. columns, Pk of 2 Requires holder 59660-U, sold separately	2	2.1	51104AST
Guard column kit for 4.6 mm I.D. columns. Contains holder, one cartridge, 2" x 1/16" tubing, two nuts, and two ferrules	2	4	51107AST
Replacement guard cartridges for 4.6 mm I.D. columns, Pk of 2. Requires holder 59660-U, sold separately	y 2	4	51106AST
Replacement guard cartridge for 10 mm I.D. columns. Requires holder 567499-U, sold separately	1	10	51108AST
Replacement guard cartridge for 21.2 mm I.D. columns. Requires holder 581392-U, sold separately	1	21.2	51109AST

#### **Guard Cartridge Holders**

Description	Cat. No.
Holder for 2 cm x 2.1 mm & 4 mm I.D. Guard Cartridges	59660-U
Holder for 1 cm x 10 mm I.D. Guard Cartridges	567499-U
Holder for 1 cm x 21.2 mm I.D. Guard Cartridges	581392-U

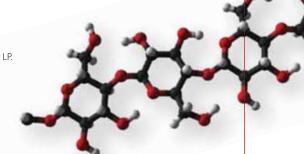
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