

Application Note 186

Astec CHIROBIOTIC CSPs for Fast Chiral LC-MS Analysis

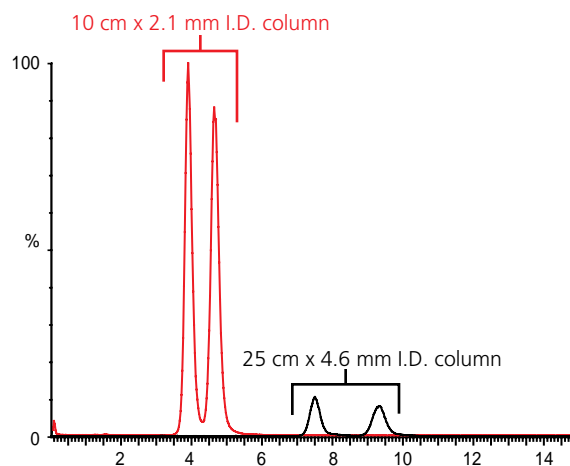
Astec CHIROBIOTIC™ CSPs (chiral stationary phases), based on macrocyclic glycopeptides covalently bonded to pure silica, often show enantioselectivity in polar organic solvents containing volatile ionic modifiers. These mobile phases are ideal for fast clinical applications and other chiral LC-MS uses, especially when the columns are short with narrow I.D.

Astec CHIROBIOTIC CSPs offer several different types of molecular interactions and separate enantiomers of many analyte classes. Of particular interest are ionic interactions. These permit operation in the polar ionic mode, which is preferred for LC-MS because its mobile phases typically comprise high percentages of methanol or acetonitrile containing low concentrations (1 to 15 mM) of volatile acids, bases or salts, such as ammonium acetate or ammonium formate. Additionally, the polar ionic mode often yields more efficient analyte ionization and correspondingly improved LC-MS sensitivity compared to reversed-phase systems.

To maximize laboratory throughput, HPLC analyses should provide the needed resolution and sensitivity in the shortest amount of time. Column configuration can be leveraged to achieve this objective. Examples of the improvement in speed and sensitivity gained by using short columns with narrow internal diameter (I.D.) are shown in Figures 1 and 2. Figure 1 compares the separation of fluoxetine enantiomers on a traditional 25 cm x 4.6 mm I.D. column to a 10 cm x 2.1 mm I.D. column, both packed with 5 μm Astec CHIROBIOTIC V2. The flow rate was adjusted to obtain the same linear velocity on both columns. The improvements in both speed and sensitivity are readily apparent. Because column efficiency is directly proportional to column length, the shorter column provides fewer theoretical plates, which resulted in lower resolution. However, Figure 2 shows that by decreasing the flow rate on the short column, equivalent resolution with dramatically improved sensitivity was obtained. The lower flow rates also permit direct connection to the MS without flow splitting.

Figure 1. LC-MS of Fluoxetine Enantiomers on Astec CHIROBIOTIC V2: Fast, Sensitive Analysis on Short, Narrow I.D. Columns

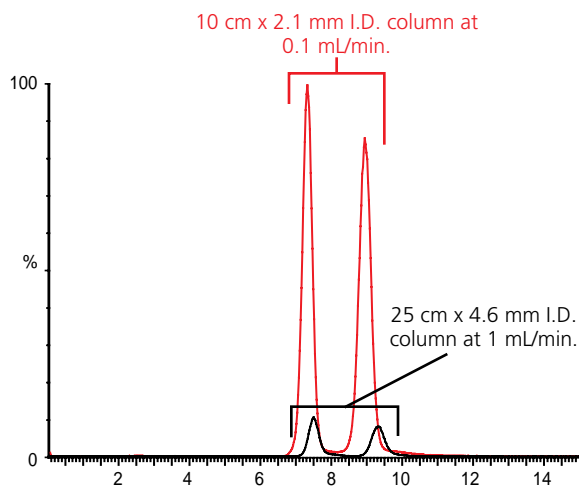
column: Astec CHIROBIOTIC V2, 25 cm x 4.6 mm (black) or 10 cm x 2.1 mm I.D. (red), 5 μm particles
 mobile phase: 10 mM ammonium formate in 10:90 v/v water:methanol
 flow rate: 1.0 (black) or 0.2 (red) mL/min.
 temp.: ambient
 det.: +ESI, m/z 310
 injection: 2 μL
 sample: 5 μg/mL in methanol



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Figure 2. LC-MS of Fluoxetine Enantiomers on Astec CHIROBIOTIC V2: Use Column Dimensions and Flow Rate to Enhance Sensitivity without Loss of Resolution

column: Astec CHIROBIOTIC V2, 25 cm x 4.6 mm (black) or 10 cm x 2.1 mm I.D. (red), 5 µm particles
 mobile phase: 10 mM ammonium formate in 10:90 v/v water:methanol
 flow rate: 1.0 (black), 0.1 (red) mL/min.
 temp.: ambient
 det.: +ESI, m/z 310
 injection: 2 µL
 sample: 5 µg/mL in methanol



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Ordering Information

Description	Cat. No.
Astec CHIROBIOTIC V2, 10 cm x 2.1 mm I.D., 5 µm particles	15018AST
Astec CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles	15024AST
Guard column, Astec CHIROBIOTIC V2, 2 cm x 1 mm I.D., 5 µm particles (to protect 2.1 mm I.D. columns). Does not require separate holder	15101AST
Guard cartridge, Astec CHIROBIOTIC V2, 2 cm x 4 mm I.D., 5 µm particles (to protect 4.6 mm I.D. columns). Requires holder	15100AST
Holder for 2 cm x 4 mm I.D. guard cartridges. Stand-alone style	21150AST
Holder for 2 cm x 4 mm I.D. guard cartridges. Direct-connect style	504254

This is a listing of the products discussed in this study. For the complete Astec CHIROBIOTIC product line, please visit sigma-aldrich.com/astec.

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