



## LARIHC Series HPLC Column Operating Instructions

### Introduction to LARIHC Series

#### **LARIHC CF6-P**

This new chiral stationary phase (CSP) was developed as an alkyl derivatized cyclofructan 6 chiral stationary phase. It has demonstrated pronounced enantioselectivity toward all types of primary amines, such as amino alcohols, amino esters, and amino amides. Baseline separation was achieved for simple aliphatic racemic amines that contained no other functionality. Unlike all current crown ether chiral stationary phases, this new column works more effectively with organic solvents and supercritical fluids. It also appears to have great capabilities for preparative-scale separations. No other existing phase can efficiently separate primary amines as well as the LARIHC CF6-P phase. A recent study showed that this phase alone can separate 93% of tested racemic primary amines.

#### **LARIHC CF6-RN**

This CSP utilizes R-naphthylethyl-functionalized cyclofructan 6 as the chiral selector. This new CSP shows excellent enantioselectivity toward various types of analytes, including acids, secondary and tertiary amines, alcohols, and many neutral compounds. Its covalent-bonded nature allows the use of all common organic solvents creating a wide range of compound types that can be separated. This phase can be operated in all three modes. However, better resolution was obtained in the normal phase mode (due to higher selectivity) allowing for the potential for preparative separations. This phase is an excellent choice for enantiomers that are not primary amines.

#### **LARIHC CF7-DMP**

This CSP consists of 3,5-dimethylphenyl functionalized cyclofructan 7. This phase provides excellent chiral recognition toward a broad variety of compounds. This phase can be used in all three modes of operation, but like other aromatic derivatized columns, it works most effectively in the normal phase. The LARIHC CF7-DMP phase is the only commercialized cyclofructan 7 based column. Most importantly, the LARIHC CF7-DMP CSP demonstrates complementary enantioselectivity when compared to the LARIHC CF6-RN phase.



## **Operation of LARIHC Columns**

### **Column Conditioning**

LARIHC columns are shipped in IPA. For use in normal phase or polar organic solvents, the column should be rinsed with ethanol prior to equilibration with the desired mobile phase. Before use in reversed phase mode, the column should be rinsed with water. Note, the viscosity of IPA is high, so it is important to not use a flow rate greater than 0.8 mL/min (keeping the column pressure below 300 bar) when first rinsing the column. (Note, when mobile phases containing cations are used on the LARIHC CF6-P phase, overnight storage in the mobile phase is the best way to fully equilibrate the column. A shorter equilibration time (i.e. pumping 10 column volumes) is acceptable and will yield enantiomeric separations with the same resolution as if the column was fully equilibrated. The fully equilibrated column will simply result in slightly shorter analyses times, due to a decrease in non-enantioselective retention mechanisms).

### **Mobile Phases**

LARIHC columns can be used in all three modes of operation. Typically, the polar organic mode and normal phase modes should be tested first. The following mobile phases are typically used for initial screening.

#### **a) Polar Organic Mode**

Acetonitrile/Methanol/Triethylamine/Acetic Acid (30/70/0.2/0.3)

#### **b) Normal Phase Mode**

Heptane/Ethanol/TFA (70/30/0.1)

#### **c) Reversed Phase Mode**

Acetonitrile/Buffer (30/70)

### **Column Stability (Solvents, pH, Temperature)**

LARIHC columns are bonded phases. All solvent systems are acceptable. When using buffers, it is important to maintain a pH between 3.0 and 7.5. Strong alkaline will destroy the silica chromatographic support. Strong acid will degrade the chiral selector. Changes in temperature may be used as a means of optimization. LARIHC columns have been operated at 0°C and 40°C without any loss in column performance.

### **Column Storage**

For short term storage, acetonitrile, methanol, or ethanol are acceptable solvents. For long term storage the use of IPA is recommended. Note, for complete equilibration with mobile phases containing cations, columns may be stored in the mobile phase overnight.