

Frequently Asked Questions

ABOUT PIRKLE-TYPE CHIRAL STATIONARY PHASES AND COLUMNS FROM REGIS

Throughout the past 20 years, the Sales and Technical persons at Regis have fielded hundreds of different questions related to our Chiral Stationary Phases (CSP's). Listed here you will find some of the frequently asked questions. By no means is this a complete list, if you have questions regarding chiral chromatography, please feel free to contact Regis directly or pass your question on through one of our distributors.

What is the pressure rating of your columns?

All analytical (25cm x 4.6 mm i.d.) and semi-preparative (25cm x 10.0 mm i.d.) columns manufactured by Regis can tolerate pressures up to 6000 psi. Larger columns will tolerate 3000 psi. It is very important not to exceed the maximum pressure rating for any HPLC column as you may disrupt the integrity of the silica bed and destroy the column.

Can Regis columns be reversed?

Yes, all columns packed by Regis are fully reversible. In fact, Regis was the first column manufacturer to sell a fully reversible HPLC column. It is recommended to reverse your column frequently. This helps keep the frit surface from becoming clogged with undissolved sample or particulates in the mobile phase, thus extending the column life.

What is the pH range of your columns?

All of Regis' Chiral phases are bonded on silica. The recommended pH range is 2.5 to 7.5. Limited usage outside of this pH range can be tolerated, but it has been proven that extended usage outside of the range will decrease column life.

Can your columns be used in normal and reversed-phase solvents?

Yes, all Pirkle-Type Chiral HPLC columns can be used in *BOTH* normal and reversed-phase solvents. Generally, the Pirkle-Type CSP's will give better separations by using them with normal-phase solvents. There are numerous examples, however; where separations with reversed-phase solvents will outperform those with normal-phase solvents.

Can I use the same column for reversed-phase and normal-phase solvent systems while doing method development?

Yes, just make sure you completely flush out the column with a miscible solvent such as IPA or ethanol. We recommend at least 20 column volumes.

How long does it take your columns to equilibrate?

The column should equilibrate after about 20 column volumes. When you are switching from normal to reversed-phase solvent systems and vice-versa, flush the column with a miscible solvent for 20 column volumes. It should take another 20 column volumes to equilibrate. The equilibration volumes may vary depending on the composition of the mobile phase.

What type of silica do you use?

Regis exclusively uses Exsil® for our 5-micron material and Kromasil, for 10 and 16-micron material. Both brands of silica are 100 angstrom in pore size.

Do you always need a modifier in the mobile phase?

No modifiers are usually needed for initial method development. Modifiers can be used to improve peak shape and resolution when the samples are extremely basic or acidic in nature. Acetic acid or ammonium acetate are recommended for acidic compounds, and triethylamine, diethylamine or ammonium acetate are recommended for basic compounds. Usually 0.1% of modifier is all that is required. *Note: Although TFA may be used as a modifier, its use should be limited. Acetic acid usually works as well as TFA.*

Can I use your columns for SMB chromatography and SFC?

Yes, many analytical and preparative chromatographers use Pirkle-Type Chiral columns in both SFC and SMB. Special hardware is necessary for certain column dimensions.

What is the difference between Whelk-O 1 and Whelk-O 2?

Although the Whelk-O 1 and Whelk-O 2 both share the same Chiral selector, they have distinct differences. The Whelk-O 1 is monofunctionally bonded to silica and the Whelk-O 2 is trifunctionally bonded. The Whelk-O 2 was designed to tolerate strong acidic modifiers such as TFA. The Whelk-O 2 was designed for preparative use and is not available on 5-micron silica. Due to the fact the Whelk-O 2 is a trifunctional bond, coverage on the silica will be less than with Whelk-O 1. This decrease in the actual number of bonded sites will decrease selectivity and not allow for exact reproducibility of a method developed on a Whelk-O 1 column.

Does my compound need an aromatic ring to achieve separation on a Pirkle-Type Chiral column?

In most cases (not all), yes. Chiral recognition occurs at binding sites. The potential π - π interaction that can occur between the aromatic rings on the Chiral selector and the aromatic ring on the sample is a major factor in achieving selectivity. Binding does occur at other sites such as acidic sites, basic sites and steric interaction sites—this is why you do not always need a ring—but by far, the π - π interaction is the major binding site.

Can I use the Pirkle-Type Chiral columns in polar organic mode?

Yes, even though the success rate is very poor, you can use the columns in polar organic mode. We do not recommend dedicating a slot in your method development station for a Pirkle-Type Chiral column if you are exclusively running in polar organic mode. Add another Pirkle-Type column to your normal-phase system to achieve a higher success rate.

What sampling loading can I expect from Pirkle-Type Chiral HPLC columns?

The typical loading range – with relative retention's (α) greater than 1.3—is ~ 4-16 mg of sample per gram of packing. Below are typical loadings for some of the different column sizes: *Note: Factors, such as solubility, will greatly affect loading capacity.*

Analytical column, 25cm x 4.6mm, ~ 3.5 grams of packing, loading is 14-56 mg/ injection.

Semi-prep column, 25cm x 10.0mm, ~ 16 grams of packing, loading is 64-256 mg/ injection.

Prep column, 25cm x 21.1mm, ~ 72.5 grams of packing, loading is 288-1,152 mg/ injection.