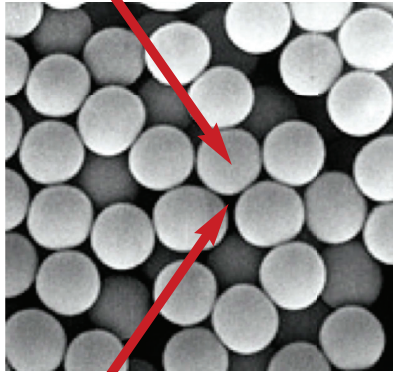


## INTERESTING COLUMN FACTS

### Why do HPLC columns plug up?

A large percentage of HPLC columns die because the back pressure builds up to

5µm Silica Gel Particle

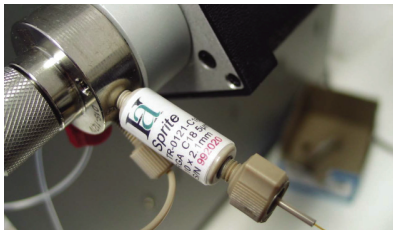


0.75µm Extra-Particle Flow Path

levels that exceed the instrument's practical limits. Most chromatographers (and many column manufacturers) believe this is because the inlet frit plugs up with particles. Actually, it is usually the packed-bed that becomes plugged by smaller than 1 µm particles that pass through in-line filters and the 2µm inlet frits. The SEM above illustrates the relative sizes of particles and pores. Higgins Analytical offers a wide variety of guard cartridges that address this problem.

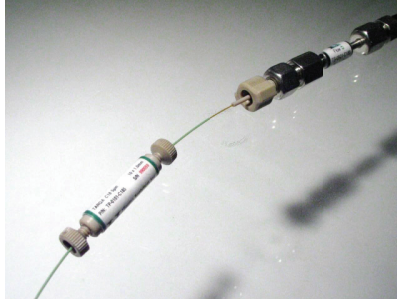
### SPRITE is Designed to Operate in Either Flow Direction

A SPRITE is made of PEEK and titanium and can be connected directly to a sample injection valve or directly to a mass spectrometer's source inlet.

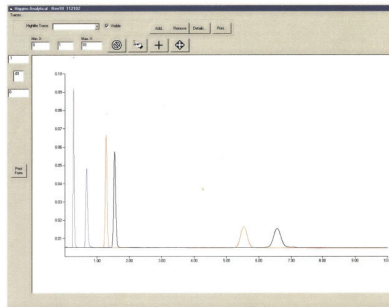


### Using a PICCOLO as a Microbore Guard Column

PICCOLO columns are normally used as trap columns but since they are manufactured with the same techniques



we use to pack microbore HPLC columns, they are very efficient. Their performance makes them ideal for use as short microbore HPLC column or as a guard for placed in front of another analytical microbore column. The chromatograms in the figure below illustrate typical performance when used in these unconventional modes.



Piccolo Column performance as an Analytical Column and as a Guard Column

Blue Trace:  
10x1mm Targa C18 5µm Piccolo Guard Column alone (P/N TP-0101-C185)

Red Trace:  
50x1mm TARGA C18 5µm Analytical Column (P/N TS-0501-C185)

Black Trace:  
10x1mm Guard coupled to 50x1mm Analytical Column

Peak #1: Dimethyl Phthalate Peak #2: Fluorene  
60% MeCN/water, 50µL/min  
System Variance ~6µL<sup>2</sup> at 50µL/min

### The Role of Column Length in Gradient Separations

Changes in column length have a direct and linear effect on theoretical plates and analysis time in isocratic separations. Column length plays a less important role, however, in gradient techniques. The three chromatograms below illustrate how well packed short HPLC columns yield very similar peptide separations under identical gradient conditions.

For this study, the 2.1mm I.D. HAISIL 300 C18 5µm columns ranged from 250 to 100mm in length. While solvent and analysis time savings may be minimal, column cost savings can be significant when using as short a column as possible. Short columns also exhibit lower pressure at high flow rates.

