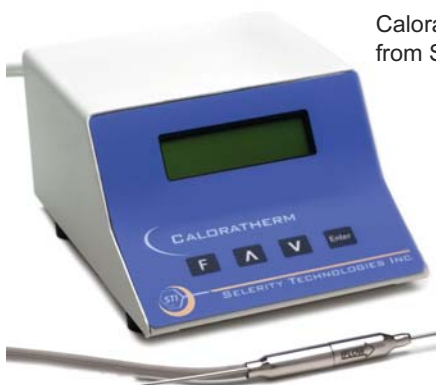


Using Theory and Empirical Data to
Optimize HPLC Systems for Speed

Users Guide for Ultra-Fast™ Kit
from GL Sciences, Inc., allowing conventional
HPLC systems to yield extremely fast
chromatography



Caloratherm active solvent preheating unit
from Selerity Technologies, Inc.

GL Sciences' Inertsil HPLC Columns



SELERITY TECHNOLOGIES, INC.



GL Sciences | *INERTSIL*

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4733 Torrance Boulevard, Suite 255, Torrance, California 90503
Tel 310.265.4424 Fax 310.265.4425 , www.inertsil.com



YOU DON'T NEED A NEW HPLC SYSTEM TO GET **ULTRA-FAST** SEPARATIONS.

LET US SHOW YOU HOW TO USE OUR ULTRA-FAST KIT TO GET ALL THE SPEED YOU NEED.

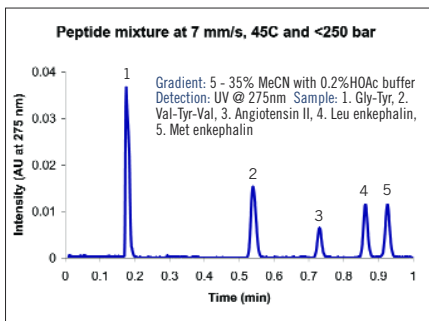
You don't have to change very much about your standard, run-of-the-mill gradient HPLC systems to achieve sub-minute separations with peaks widths under 1.0 seconds.

In other words, you don't need to go out and spend a fortune on one of the new, ultra high-pressure systems that are currently in vogue to meet the increases in productivity demanded by today's results oriented environment. And even if your budget allows you to upgrade to a new, ultra high pressure system, better control of the operational parameters using GL Sciences' Ultra-Fast Kit™ can result in further dramatic improvements in chromatography and speed.



The fact is that the critical elements of a good chromatographic separation remain the stationary phase, the mobile phase, good mass transfer and minimized system dead volume. GL Sciences' Ultra-Fast Kit provides all these critical elements to success.

Peptide Mixture on Inertsil 3 micron ODS-3, 50 x 4.6mm,
5 mL/min, 45°C, at less than 3600 PSI on a standard HPLC



Caloratherm active solvent preheating
unit from Selerity Technologies, Inc.



GL SCIENCES' ULTRA-FAST KIT CONSISTS OF:

- Two GL Sciences' **Inertsil** high-speed 3 micron 50 x 4.6 or 50 x 2.1mm columns
- Caloratherm eluent heater from Selerity Technologies, Inc
- Optimized tubing and fittings to minimize dead volume
- Detailed instructions and a thorough explanation of the critical chromatographic parameters that make Ultra-Fast separations possible.



GL Sciences

As Seen in LCGC Magazine in February,
March, and April 2007

The following document is intended to be a simple guide on transforming your existing liquid chromatographs (LC) into high throughput systems at minimal costs. The benefit of doing this can mean up to a 5-fold decrease in analysis time. As the demands on your time increase, fast, quality analyses become essential, but at what cost?

Herein is described some of the wonderfully insightful work of Dr. Mark Hayward and his colleagues, who realized that standard HPLC systems could operate much faster if only a vendor(s) would pay attention to some critical details *simultaneously*. Having found the near optimum HPLC columns in GL Sciences' Inertsil ODS-3 and C8-3 phases, he persuaded the technologists at Selerity Technologies, Inc. to develop an incredibly dynamic solvent heater with the simple programming required, and the concept of the Ultra-Fast Kits was born.

Introduction

The resolution (R_s) of any two chromatographic peaks varies with particular conditions of the separation. The parameters directly related to the separation conditions are: column selectivity, peak width, and retention. The peak retention time (t_r) must be reduced to shorten the analysis, but not at the expense of resolution. Since productivity is proportional to resolution per unit time, no improvement in productivity is gained if too much resolution is lost.

Maximizing productivity (R_s/t_r), while minimizing time (t_r), requires that peak width (w) must also be minimized.

$$R_s = 2(t_{r2} - t_{r1}) / (w_1 + w_2)$$

Peak width minimization can be achieved with any existing HPLC system by paying attention to the parameters that contribute to the broadening of the peaks, i.e., dead volume, dwell volume, back pressure and temperature mismatch across the column.

Concept

Step 1 - Optimize System Plumbing:

The tubing length must be minimized before the column so that the delay time for the gradient to reach the column is as short as possible. However, larger inner diameter tubing should be used before the column to prevent unnecessary system back pressure. The amount of tubing after the column should also be minimized, in addition to downsizing the ID so that the unnecessary volume does not impact efficiency.

Tips for Optimizing Plumbing

- Standard HPLC with pressure limit approximately 5000 psi.: use 0.010" tubing before the column and 0.007" tubing after the column.
- New high pressure HPLC with pressure limits above 10,000 psi: remove original tubing used for passive preheating and replace with a short piece of 0.005"ID tubing

Step 2 - Choose the Appropriate Column Dimensions and Particle Size:

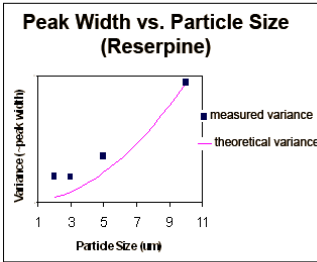
The use of smaller particles will facilitate narrow peaks under all conditions. When using smaller particles, less of an impact on the peak width is observed as the velocity is increased; therefore, it is possible to achieve the same separation with shorter columns to reduce analysis times. *However, there are always trade-offs and, in practice, particles less than 3 μ m do not produce enough reduction in peak width to offset large pressure increases, even when using optimized high pressure systems.*



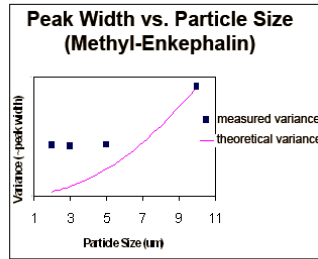
Tips for Choosing a Column

- None of the previously mentioned tips will matter without a high quality column, such as GL Sciences' Inertsil 3 micron ODS-3, C8-3, etc.
- Peak width differences of up to 5 fold can be demonstrated between top quality columns and standard commercially available columns.
- Head to head comparisons are the only way to optimize this parameter. Test new columns frequently and don't believe only one or two tests; seek low operating pressures, for which Inertsil is unique among the top HPLC column contenders.
- 50 mm lengths with 3 μ m particles provide a good balance between speed and resolution (resolution is similar to 150 mm lengths with 5 μ m particles).
- Use of columns that offer excellent efficiencies at low operating pressure, good mass transfer, acceptable lifetime and batch to batch reproducibility. Again, GL Sciences' Inertsil is the perfect choice to meet these demands.

**With a Well Chosen Stationary Phase
Smaller Particles do not Yield Narrower Peaks**



**Acetonitrile soluble compounds:
Peak widths level out at 3µm**

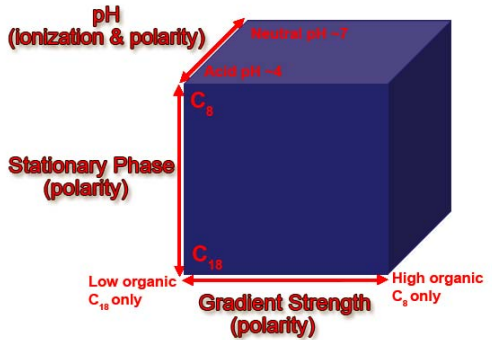


**Water soluble compounds:
Peak widths level out at 5µm**

Step 3 - Choose the Appropriate Mobile Phase Composition:

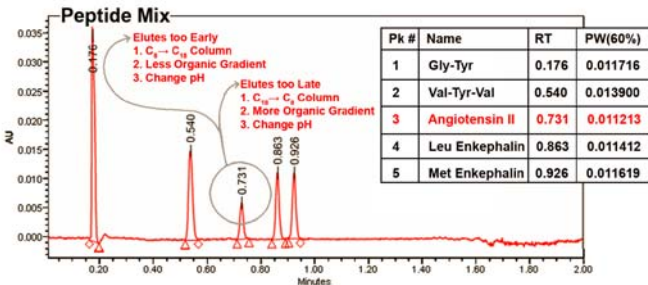
The mobile phase choice should be selected based on pH control, MS ionization and/or viscosity and elution strength. The goal when choosing the mobile phase is to use a mobile phase composition and gradient rate that elutes the analyte with the narrowest peak width in the middle of the chromatogram. To achieve this goal, columns with different stationary phases, mobile phases with different pH's, and the amount of organic in the mobile phase can be used. To see an illustration of the parameters that can be changed see the 2x2x2 Polarity Matrix .

2x2x2 Polarity Matrix



Goal for Mobile Phase:

Smallest Peak Widths Should be the Peaks Eluting in the Middle of the Chromatogram



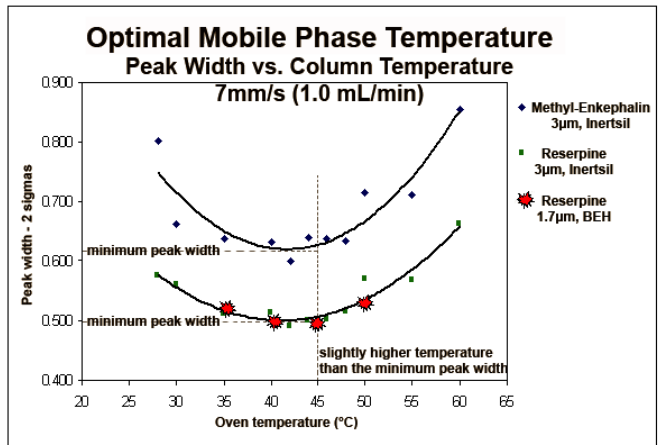
Tips for Choosing a Mobile Phase

- Acetonitrile (residue/UV grade, high purity) with low viscosity & good eluting strength.
- High purity CH_3COOH (acid pH) and NH_4COOH (neutral pH) for buffers in ultra high quality H_2O for pH control and compatibility with MS ionization (not recommended if one must use low UV wavelength detection).

Step 4 - Preheat the Mobile Phase with an Active Preheating Unit:

It is now well recognized that temperature impacts peak efficiency, and if the temperature differential between the column and the incoming mobile phase is too large, band broadening results. This can occur even at 40°C . The ability to accurately and reproducibly control the column temperature at relatively high levels ($60^\circ+$) is critical to promoting the sample diffusion rates required to achieve fast separations. Therefore, it is necessary to use an active pre-heater that has the ability to sense the amount of power that is needed to hold the mobile phase at a constant temperature throughout the analysis when the heat capacity of the mobile phase rapidly changes. The best results are found when the incoming mobile phase temperature is not more than 5°C different from that of the column temperature. The only column heating product available that is capable of meeting these demands is the Caloratherm™ solvent preheater invented by Selerity Technologies, Inc., which has an exception ability to change it's power output at the rate determined most effective by the chromatographer.

Caloratherm active solvent preheating unit from Selerity Technologies, Inc.



Tips for Optimizing the Mobile Phase Temperature

- Use a Selerity Caloratherm™ mobile phase preheater.
- Measure the peak width at different temperatures near the column set point and choose the optimum temperature, which occurs slightly toward the higher temperature side of the minimum peak width.

Step 5 - Increase the Flow Rate :

Increasing the flow rate and adjusting the mobile phase temperature is where this technique significantly deviates from standard practices. Set the flow as high as possible without going beyond 75% of the maximum available pressure, then adjust the mobile phase temperature so that the peak width is optimized.

Tips for Optimizing the Flow Rate

- Choose a flow rate based on pump pressure. The preferred pressure is approximately 50-60% of the maximum operating pressure and we recommend limiting the pressure to 75% of the absolute maximum.
- Standard HPLC conditions: the optimum flow is 5mL/ min with a 4.6 X 50 mm column using 3-3.5 μm particles and 45° C.
- High pressure HPLC conditions: the optimum flow is 2mL/ min with a 2.1 X 50 mm column and with 3-3.5 μm particles and 60°C

How to Convert Systems for Fast HPLC

Example - Waters' Ultra Performance LC™:

1. Remove high mass filter and preheater unit consisting of a block heater and tubing.



2. Slide the Caloratherm preheating unit onto a new piece of stainless steel tubing that is 0.005"ID and 20cm in length. Use the appropriate nuts and ferrules and pay close attention that the tubing is seated firmly inside of the fitting.



3. Install tubing with the Caloratherm between the column and injector; set the temperature at 60°C.

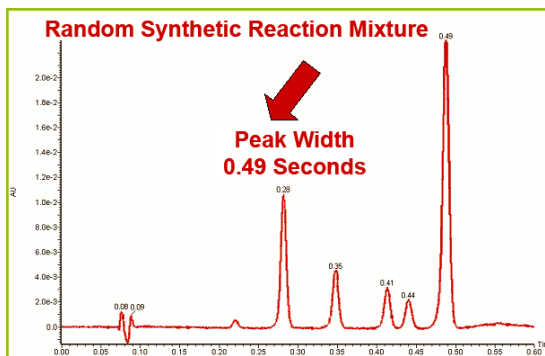


4. Column: **Inertsil 3 μ m ODS-3, 50 x 2.1mm**

5. A flow rate of 2.0 mL/min, and determine the gradient conditions to optimize your separation.



Example - Waters' Aquity™ System:



Waters Aquity™ UPLC
InertSil C18 Column 3 μ m, 2.1 X 50mm
(14mm/sec) 2 mL/min., 60°C

Example - Waters' Alliance™ Configured with Multiple Columns

1. Remove existing tubing and replace with 0.010" ID before column and 0.007" ID after column.

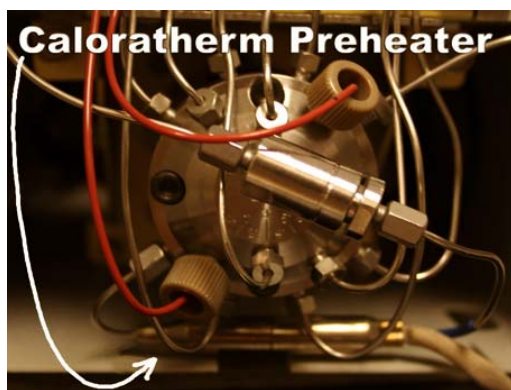


2. Remove the existing sample loop that has a 50 μ L volume and replace with a sample loop that has a 20 μ L volume (40cm of 0.010" ID tubing).

3. Modify the Alliance™ oven to provide uniform heating by removing the back panel and re-routng the tubing through the metal plate.

4. Install Caloratherm before injection valve, set temperature at 45°C.

(see next page)



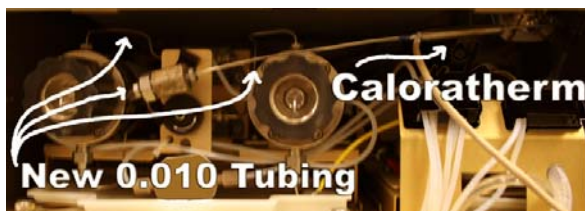
Example - Waters' Alliance™ Configured with Multiple Columns (continued)

5. In this case, the system is used with 50 x4.6mm GL Sciences' Inertsil ODS-3, 5 µm columns, as shown at right.

6. Use a 5mL/min flow rate.

Example - Waters' Alliance™ Configured with one column and minimal volume:

1. Remove tubing before injection process. Replace with 0.010" X 30cm tubing. Insert 0.010" X 30cm tubing fitted with a Caloratherm mobile phase preheater between the pump and injection valve.



2. Remove tubing after injection process, before column. Replace with 0.005" X 20cm tubing fitted with a second Caloratherm.



(continued on following page)



Waters' Alliance™ HPLC Configured with One Column and Minimal Volume (continued)

3. Remove existing sample loop. Replace with tubing that is 0.005" X 30cm (approximately 4 μ L volume).



New Sample Loop

4. Place the column inside of an Analytical Sales™ column heater

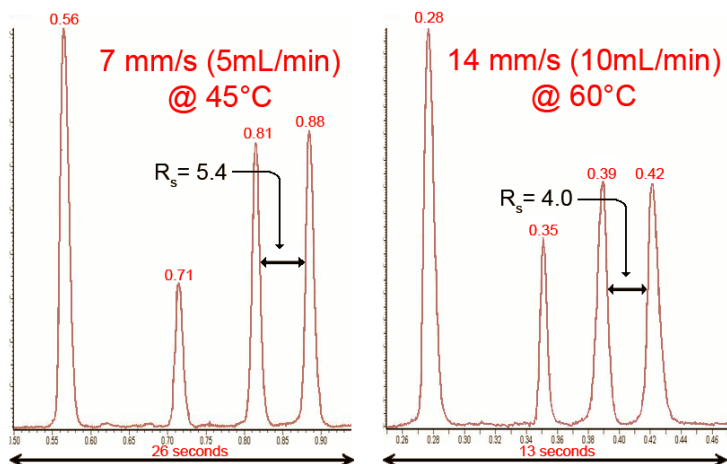


5. Here we use 3 μ m particles in a 50 x 4.6mm configuration.

6. Use a 5mL/min flow rate.

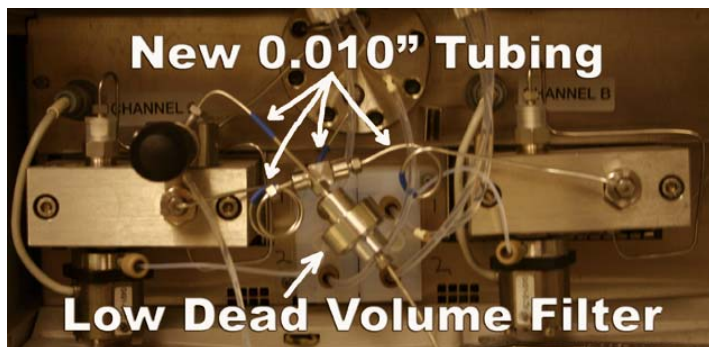
Scalable Productivity

74% conservation of resolution in half the time



Example - Agilent 1100 Configured as an Open Access System

1. Bypass high volume mixing chamber.



2. Install a 2.0 μm in-line low dead volume filter obtained from Agilent Technologies.

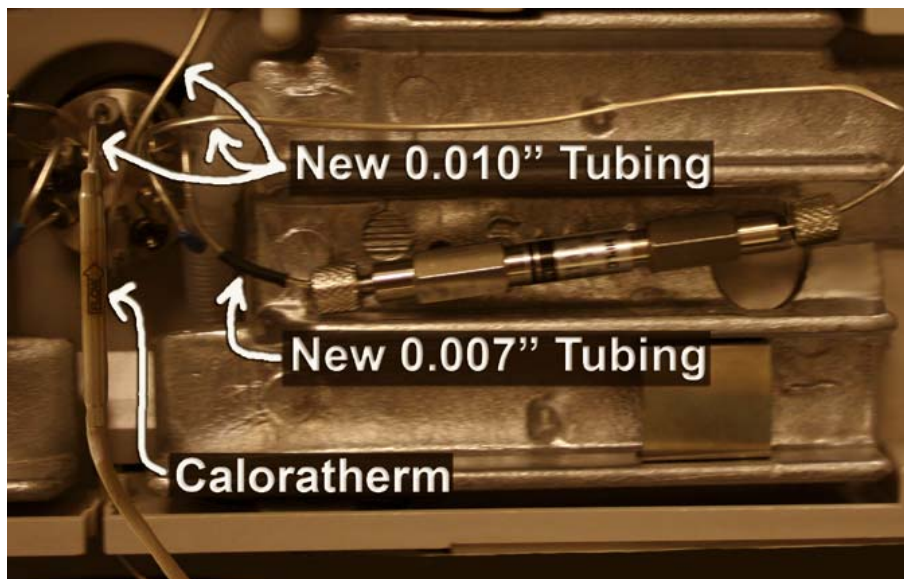
3. Remove existing tubing and replace with 0.010" stainless steel tubing before the column and 0.007" tubing after the column. When adding the new tubing, bypass the thermal equilibration tubing that is embedded within the column



(continued on following page)

Example - Agilent 1100 Configured as an Open Access System (cont.)

4. Install a Caloratherm mobile phase preheater before the sample loop on a new piece of 0.010" tubing.



5. Remove the existing sample loop and replace with a sample loop that has a 20 μ L volume (40cm of 0.010"ID tubing).

6. Add the Agilent brand stainless steel mesh plate to column heater. This plate is needed to insulate the column for uniform heating.



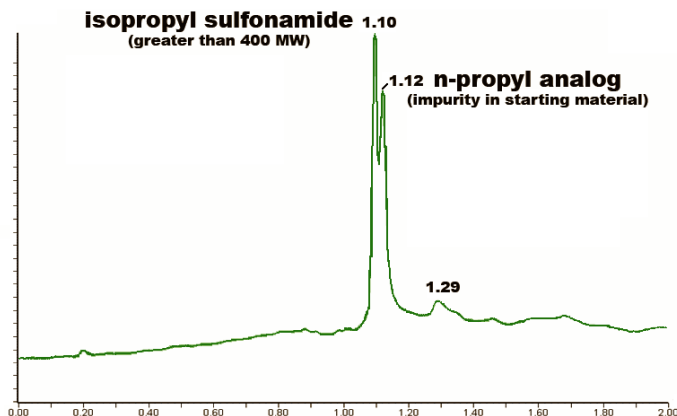
7. Use 4.6 X 50mm, 5 μ m particle size columns.

8. Use a 5mL/min flow rate.

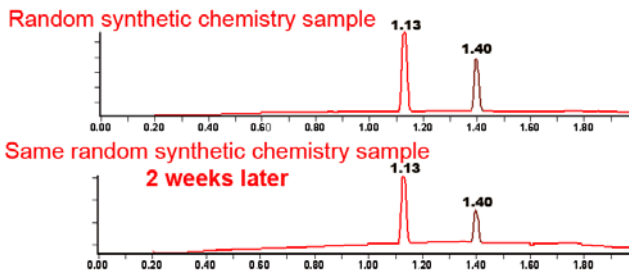
Results from Agilent 1100 Configured as an Open Access System

Compound Purity Example High Efficiency - Fast!

Information Gained: NMR Data Provides the Answer



Reproducibility From a Ten-Year Old Open Access Agilent System



Acknowledgements:

Mark J. Hayward and Qing P. Han, Lundbeck Research USA,
215 College Road, Paramus, NJ 07652

Jody Clark, Selerity Technologies, Inc., 2484 West Custer Road,
Salt Lake City, UT 84104, jodyclark@selerity.com,
Phone (801)978-2295

GL Sciences' Inc. Ultra-Fast Kit Ordering Information

GL SCIENCES ULTRA-FAST KITS CONSIST OF THE FOLLOWING BASIC ELEMENTS, WITH A VARIETY OF ADDITIONAL COMPONENTS THAT CAN BE ADDED TO MEET ANY PARTICULAR NEED:

- Two GL Sciences' Inertsil high-speed 3 micron 50 x 4.6, 2.1, 1.5, or 1.0 ID columns
- Selerity Technologies' Caloratherm eluent heater
- Tubing and fittings kit with the perfect end finishing essential to zero dead volume connections along with the nuts and ferrules necessary to plumb the system
- Detailed instructions and a selection of publications from Dr. Hayward offering the practical and theoretical underpinnings that allow these ultra fast HPLC separations to happen.

<u>Order No.</u>	<u>Description</u>	<u>Price</u>
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GL Sciences | *INERTSIL*

GL Sciences, Inc., USA

Contact: 4733 Torrance Boulevard, Suite 255, Torrance, California 90503
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The collage features the GL Sciences logo at the top left. The central and right portions show various HPLC components: several Inertsil HPLC columns of different lengths and diameters, metal fittings and nuts, a syringe, and a fritted disk. The bottom of the collage has a dark blue background with the text 'HPLC COLUMNS' in large, light blue letters. A small table of product specifications is visible in the bottom right corner of the collage.

Part No.	Part Description
INERTSIL 3000	3 µm, 50 x 4.6 mm ID
INERTSIL 3000	3 µm, 50 x 2.1 mm ID
INERTSIL 3000	3 µm, 50 x 1.5 mm ID
INERTSIL 3000	3 µm, 50 x 1.0 mm ID
INERTSIL 3000	3 µm, 100 x 4.6 mm ID
INERTSIL 3000	3 µm, 100 x 2.1 mm ID
INERTSIL 3000	3 µm, 100 x 1.5 mm ID
INERTSIL 3000	3 µm, 100 x 1.0 mm ID
INERTSIL 3000	3 µm, 150 x 4.6 mm ID
INERTSIL 3000	3 µm, 150 x 2.1 mm ID
INERTSIL 3000	3 µm, 150 x 1.5 mm ID
INERTSIL 3000	3 µm, 150 x 1.0 mm ID