CHROMATOGRAPHY

Hypersil GOLD™ 1.9 µm HPLC Columns Technical Guide

Optimizing Performance – Making Your Assay More Productive

- Faster Analyses
- Improved Resolution
- Enhanced Sensitivity
- Increased Peak Capacity
- Easier Method Development

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

1.9 µm Hypersil GOLD Columns

Based on high purity silica technology, along with a proprietary bonding and end-capping procedure, Thermo Scientific Hypersil GOLD™ columns offer improved chromatography and a new solution to the challenges facing your lab. The outstanding peak symmetry even for basic compounds delivered by Hypersil GOLD has already improved productivity in laboratories all over the world.

The use of sub-2 µm particles is becoming increasingly popular for applications in either High Throughput Screening (HTS) assays or in Ultra High Pressure Liquid Chromatography (U-HPLC). 1.9 µm Hypersil GOLD columns offer advantages over the more traditional systems containing 3 and 5 µm particles through:

- **Operating at higher flow rates without compromising efficiency**
- **Shorter analysis times**
- **Improvements in resolving power and sensitivity**
- **A choice of chemistries for different selectivity options**

The 1.9 µm Hypersil GOLD columns build on the recent advances in technology made with Hypersil GOLD media, and now offer a practical solution to improving laboratory throughput with your existing HPLC equipment. While 1.9 µm Hypersil GOLD columns can be used for U-HPLC applications, the narrow particle size distribution means that improved speed and resolution can still be attained while maintaining the pressure within the range of conventional HPLC systems.

This technical guide will focus on using existing HPLC equipment and how to optimize the system to get the best performance with 1.9 µm particles and harvest their benefits.

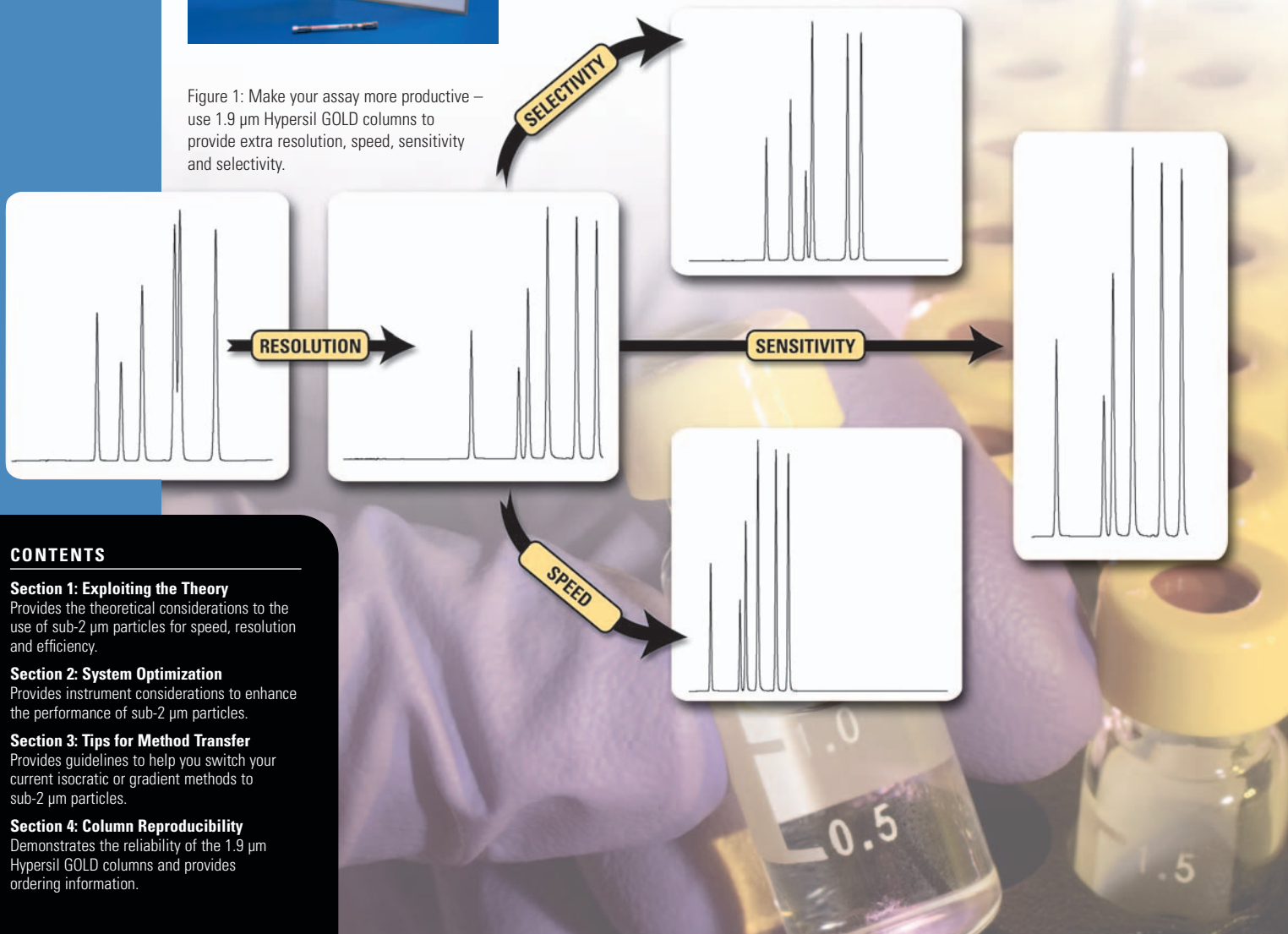

Figure 1: Make your assay more productive – use 1.9 µm Hypersil GOLD columns to provide extra resolution, speed, sensitivity and selectivity.

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Section 1: Exploiting the Theory
Provides the theoretical considerations to the use of sub-2 µm particles for speed, resolution and efficiency.

Section 2: System Optimization
Provides instrument considerations to enhance the performance of sub-2 µm particles.

Section 3: Tips for Method Transfer
Provides guidelines to help you switch your current isocratic or gradient methods to sub-2 µm particles.

Section 4: Column Reproducibility
Demonstrates the reliability of the 1.9 µm Hypersil GOLD columns and provides ordering information.

Section 1: Exploiting the Theory

The separating power of a chromatographic column can be described by the height equivalent to a theoretical plate (H), which varies with the linear velocity (u) of the mobile phase as it passes through the column, the particle size (d_p), the diffusion coefficient in the mobile phase (D_m) and is given by Equation 1:

$$H = Ad_p + BD_m/u + C(d_p^2/D_m)u \qquad (1)$$

where A, B, and C are constants relating to particle morphology, particle size and column packing.

The lower the plate height, the higher the separating power of the column, and there is an optimum linear velocity for which H is a minimum and the plate number, or efficiency, N, is a maximum. The plate height is related to the efficiency by Equation 2:

$$H = L/N \qquad (2)$$

where L is the column length.

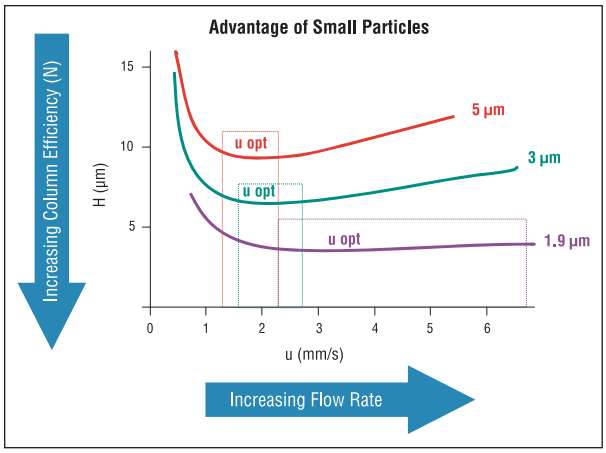


Figure 2: The Advantages of 1.9 µm Particles. The van Deemter plot highlights how columns packed with smaller particles can operate over a wider range of linear velocity and maintain higher efficiencies. This allows the use of higher flow rates, resulting in considerable improvements in speed, without loss in performance.

These equations also show that the efficiency is inversely proportional to the square of particle size.

$$N \propto 1/d_p^2 \qquad (3)$$

In short, the efficiency increases as particle size decreases.

The van Deemter curve illustrates how the limitations in column efficiency at higher linear velocities can be overcome by employing smaller particles. As the particle size is reduced, the optimum mobile phase velocity (u) is increased and the curve becomes flatter. Therefore, columns packed with smaller particles can be operated over a wider range of linear velocity while maintaining high efficiencies. This has the resultant effect of enabling the use of higher flow rates to decrease analysis time without compromising performance.

Linear Velocity and Flow Rate

The mobile phase linear velocity (u) is related to the mobile phase flow rate in the column (F) and the column cross sectional area by Equation 4:

$$u = 4F/\varepsilon\pi d_c^2 \qquad (4)$$

where ε is the volume fraction of the column between the particles and d_c is the column internal diameter.

Equation 4 tells us that in order to maintain a constant mobile phase linear velocity, the mobile phase flow rate needs to be lower for a narrower column. Figure 3 illustrates the linear velocities and flow rates for three common column internal diameters. For each of these the optimum flow rate for columns packed with 1.9 µm particles is highlighted.

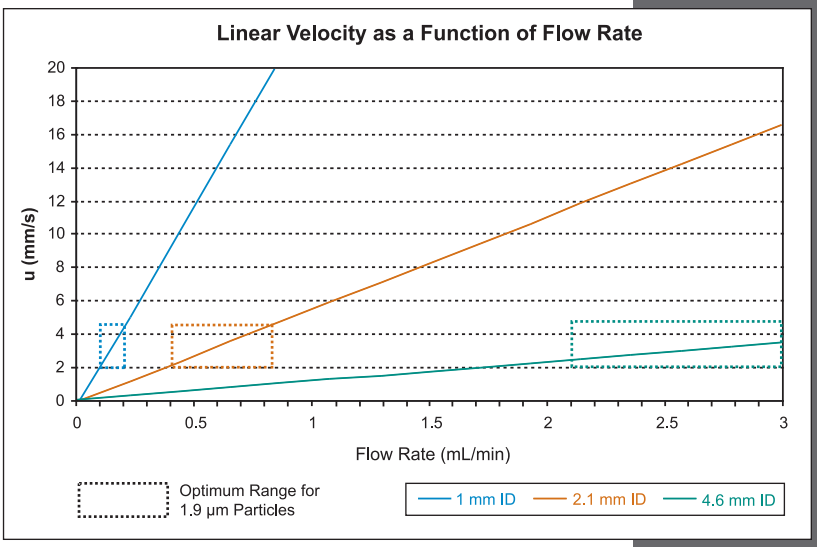


Figure 3: Optimal Flow Rates for Columns Packed with 1.9 µm Particles. As column ID decreases, the mobile phase flow rate must decrease in order to maintain a constant linear velocity.

Resolution

The aim of a chromatographic separation is to maximize resolution while minimizing analysis time. Resolution (R_s) is proportional to the square root of separation efficiency (N), as described by Equation 5 (which expresses resolution as a function of capacity factor (k), selectivity (α), and efficiency (N)). Efficiency is in turn inversely proportional to the square diameter of particle size (d_p), as discussed above.

$$R_S = \frac{1}{4}\frac{(\alpha - 1)}{\alpha}\sqrt{N}\,\frac{k}{1+k} \qquad (5)$$

Resolution and analysis time are determined by the ratio of column length to particle size. When particle size is reduced, column length can also be reduced while keeping separation efficiency constant (and therefore R_s if all other experimental conditions remain unchanged). Figure 4 illustrates this concept. For example, if 13,500 plates (green line on graph) are needed to obtain the required resolution, a 150 mm column packed with 5 µm particles would be required. However, if the particle size is reduced to 1.9 µm then only 50 mm of column is needed to obtain the same 13,500 plates. For a constant flow rate, analysis time would be reduced approximately 3-fold with this change in particle size and column length.

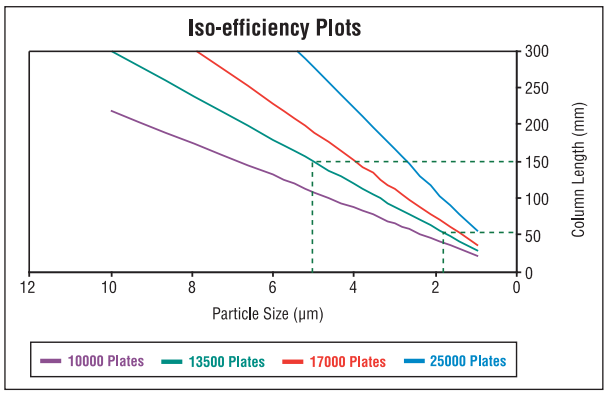


Figure 4: Iso efficiency plots. High throughput, high efficiency separations can be obtained with short columns packed with small particles.

Selectivity

Equation 5 also points out another very effective way to increase the resolution of two chromatographic bands – increasing the selectivity factor (α) by manipulating column chemistry or mobile phase composition. We offer 1.9 µm Hypersil GOLD columns in three chemistries.

- **Hypersil GOLD** gives outstanding peak shapes using generic gradients with C18 selectivity.
- **Hypersil GOLD aQ** is a C18 polar endcapped stationary phase which can be used for challenging reverse phase separations employing highly aqueous mobile phases.
- **Hypersil GOLD PFP** is a perfluorinated phenyl stationary phase which can offer alternative selectivity in reverse phase applications.

Figure 5 illustrates the difference in selectivity between the three phases.

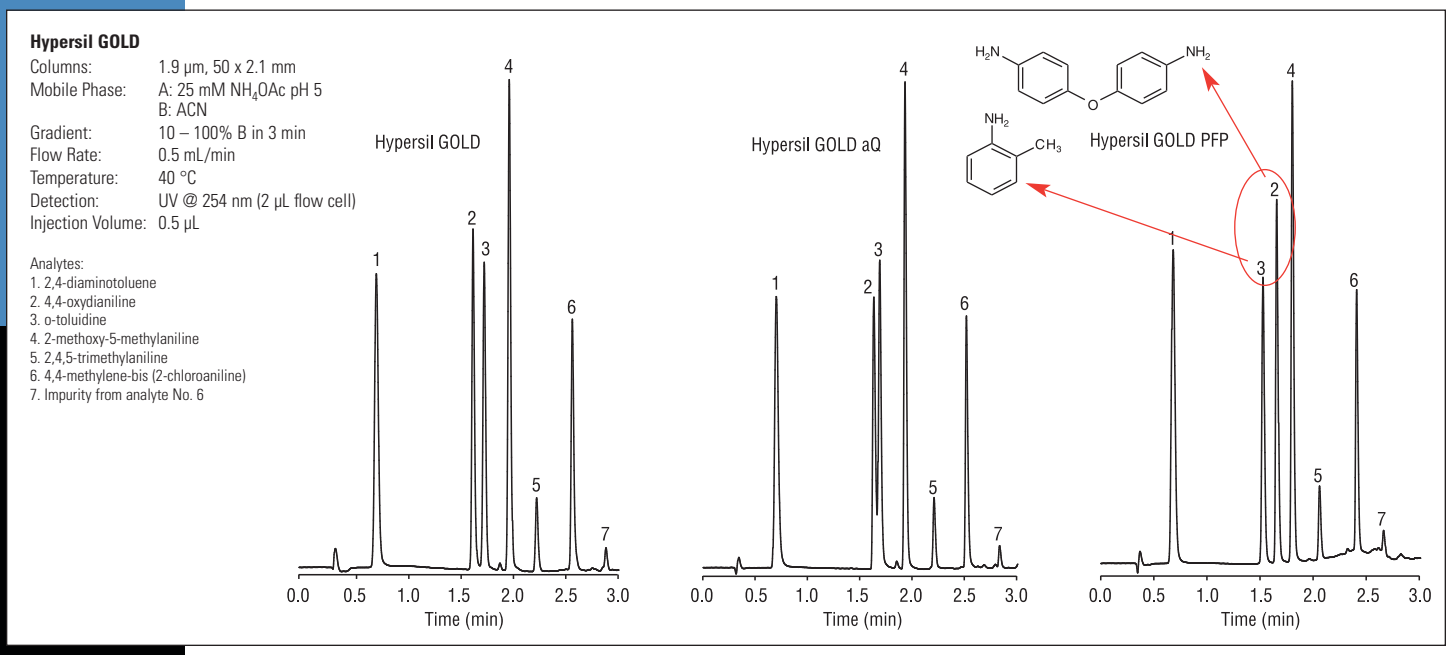


Figure 5: Effect of column chemistry (C18 selectivity, C18 polar endcapped and pentafluorophenyl) on the separation of aromatic amines. Note the change in elution order for compounds 2 and 3 for Hypersil GOLD PFP.

Sensitivity

Sensitivity or signal-to-noise ratio is related to concentration at peak apex, c_{max}, which depends on chromatographic parameters such as efficiency (N), injection volume (V_i), column length (L), column internal diameter (d_c) and capacity factor (k), as expressed by Equation 6:

$$c_{max} \propto \frac{\sqrt{N}\ V_i}{L d_c^2 (1 + k)} \qquad (6)$$

This dependence points out some ways to increase the sensitivity: reduce the length and reduce the internal diameter of the column and increase separation efficiency. This is illustrated in Figure 6 for the separation of four steroids.

Decreasing column length (Figure 6 step 1) results in a shorter run time if all other experimental parameters are kept unchanged. Note that, when decreasing the column internal diameter (Figure 6 step 2), it is necessary to adjust the mobile phase flow rate to maintain a constant mobile phase linear velocity through the column.

By decreasing the particle size (Figure 6 step 3), efficiency is increased, which leads to more sensitivity. Using smaller particles also allows us to increase the mobile phase flow rate without losing separation performance, which also increases the speed of analysis.

Hypersil GOLD (dimensions vary as shown)
Mobile Phase: H_2O/ACN (50:50) +0.1% Formic acid
Flow Rate: Varies as shown
Temperature: 25 °C
Detection: UV @ 244 nm
Injection Volume: 60 nL

Analytes:
1. Cortisone
2. 11-α-hydroxyprogesterone
3. 17-α-hydroxyprogesterone
4. Progesterone

Original Conditions	Step 1: Reducing column length	Step 2: Reducing column ID, decreasing flow rate to maintain constant linear velocity	Step 3: Reducing particle size and increasing flow rate
L: 100 mm	L: 50 mm	L: 50 mm	L: 50 mm
d_c: 4.6 mm	d_c: 4.6 mm	d_c: 2.1 mm	d_c: 2.1 mm
d_p 5 µm	d_p: 5 µm	d_p: 5 µm	d_p: 1.9 µm
Flow Rate: 1 mL/min	Flow Rate: 1 mL/min	Flow Rate: 0.2 mL/min	Flow Rate: 0.55 mL/min

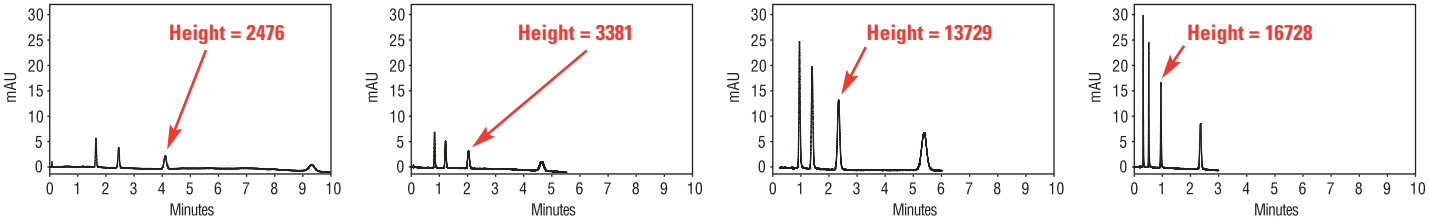


Figure 6: Improving sensitivity by reducing column length (L), column ID (d_c) and particle size (d_p). This series of chromatograms demonstrates how a step-wise decrease in column length, column ID and particle size can affect peak height, and therefore sensitivity. The parameters that have been changed in each step are highlighted in red.

Speed

The opportunity to increase sample throughput has generally been compromised by a trade-off between column dimensions and operational parameters associated with system capability. To demonstrate how the use of smaller particles in this environment gives an additional route to improve sample turnaround, a separation of seven phenone compounds performed on a 200 x 2.1 mm, 5 µm column was transferred to shorter columns packed with smaller particles to reduce analysis time. The flow rate and gradient time were adjusted to reflect the changes in column length and particle size. The 1.9 µm particles facilitate a decrease in run time from 6 to 0.5 minutes, while maintaining baseline resolution of the seven phenones, as seen in Figure 7. In the top chromatogram, the column temperature has also been increased to achieve additional speed.

Summary

Numerous experimental parameters can be manipulated to improve speed, efficiency or resolution of a chromatographic separation. However, serious trade-offs existed to achieve both until the introduction of sub-2 µm particles, such as Hypersil GOLD 1.9 µm columns. The exceptional range of optimal linear velocity that can be achieved using sub-2 µm particles allows the use of high flow rates to reduce analysis time while maintaining chromatographic efficiency. In addition, the smaller particles allow for the reduction in column length while maintaining efficiency, providing further gains in speed of analysis at high flow rates without exceeding the pressure limits of standard HPLC systems.

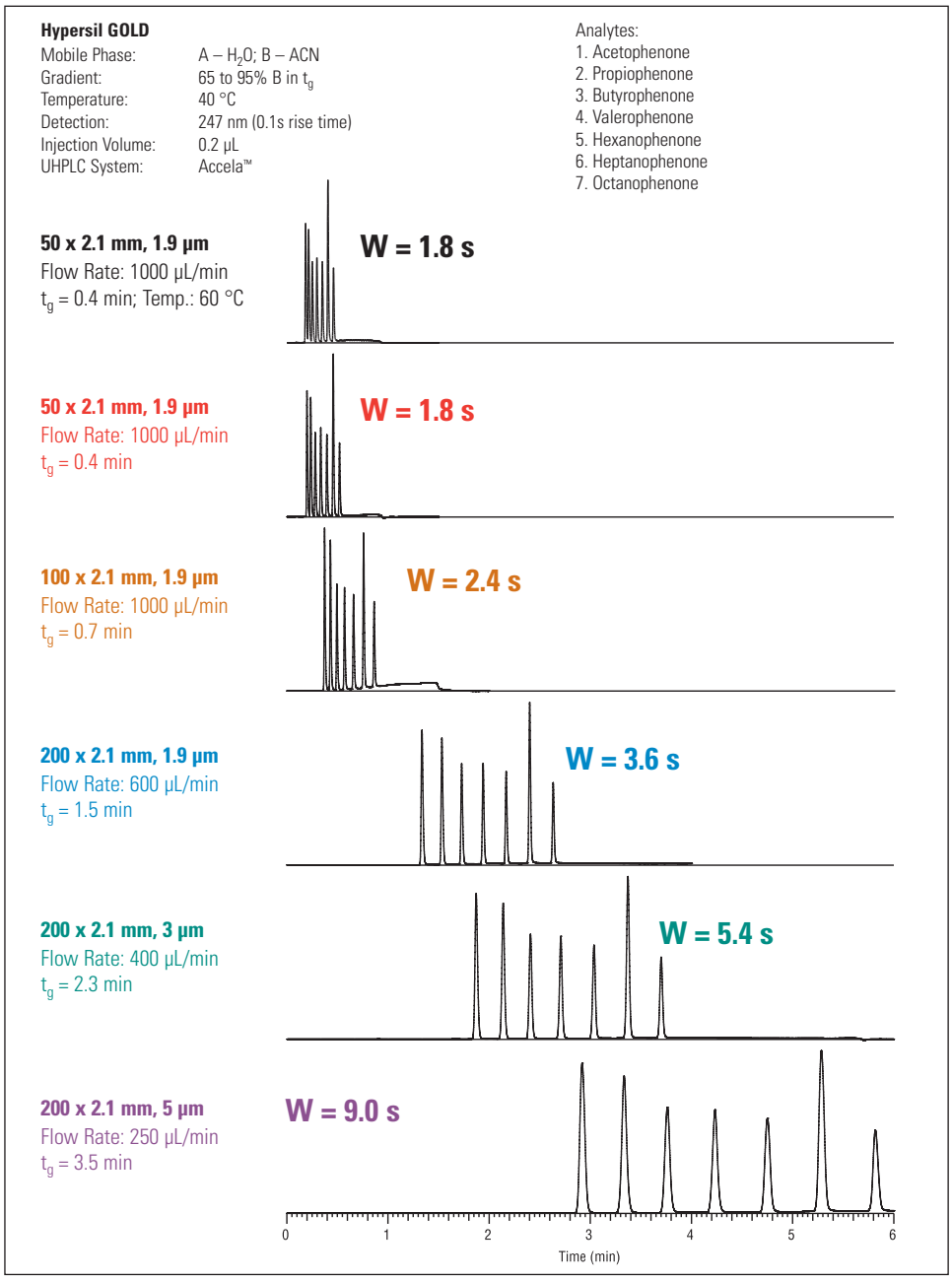


Figure 7: Effect of column length, particle size and operating conditions on run time and peak width at baseline (W).

Section 2: System Optimization

We have seen that when using columns packed with 1.9 µm particles, analyses can be performed with a high linear velocity through the column without loss in performance. To obtain the best data using fast chromatography, however, it is critical that the LC instrument system is optimized to operate under these conditions. All system components for the assay should be considered. System volume (connecting tubing ID and length, injection volume, flow cell volume in UV) must be minimized, detector time constant and sampling rate need to be carefully selected, and when running fast gradients pump dwell volume needs to be minimal.

System Dispersion

Band broadening, which has a detrimental effect on the chromatographic performance, can occur in the column, in the autosampler, in the tubing connecting the column to injector and detector and in the detector flow cell. These band broadening effects which occur in the fluid path of the HPLC instrument are volumetric effects; each contributes a variance (σ) to the width of the chromatographic band and are additive:

$$\sigma^2_{total} = \sigma^2_{col} + \sigma^2_{ext} \qquad (7)$$

where the subscripts mean: col – inside column, ext – extra column.

As a guide for good chromatography, the extra column band broadening, originating from the injector, flow cell and tubing (see Equation 8), should not exceed 10% of the total band broadening. The extra column effects are more significant for scaled down separations (column volume decreases) and for less retained peaks which have a lower peak volume. It is therefore critical to minimize extra column dispersion. Figure 8 highlights the improvements that can be made in resolution, asymmetry and efficiency by reducing the injection volume (left), the flow cell volume (center) and column to detector connecting tubing ID (right).

$$\sigma^2_{ext} = K_{inj}\frac{V^2_{inj}}{12} + K_{cell}\frac{V^2_{cell}}{12} + \tau^2F^2 + \frac{r^4_c \cdot l_c \cdot F}{7.6 \cdot D_m} \qquad (8)$$

In addition to the volumetric effects, the time constant of the detector (τ, response rate) and the scan rate may also contribute to the broadening of the peak, and should be considered.

Detector Time Constant

The central term covering flow cell volume in Equation 8 also shows that dispersion in the detector is dependent upon the detector time constant τ. Reducing the time constant will reduce the observed dispersion.

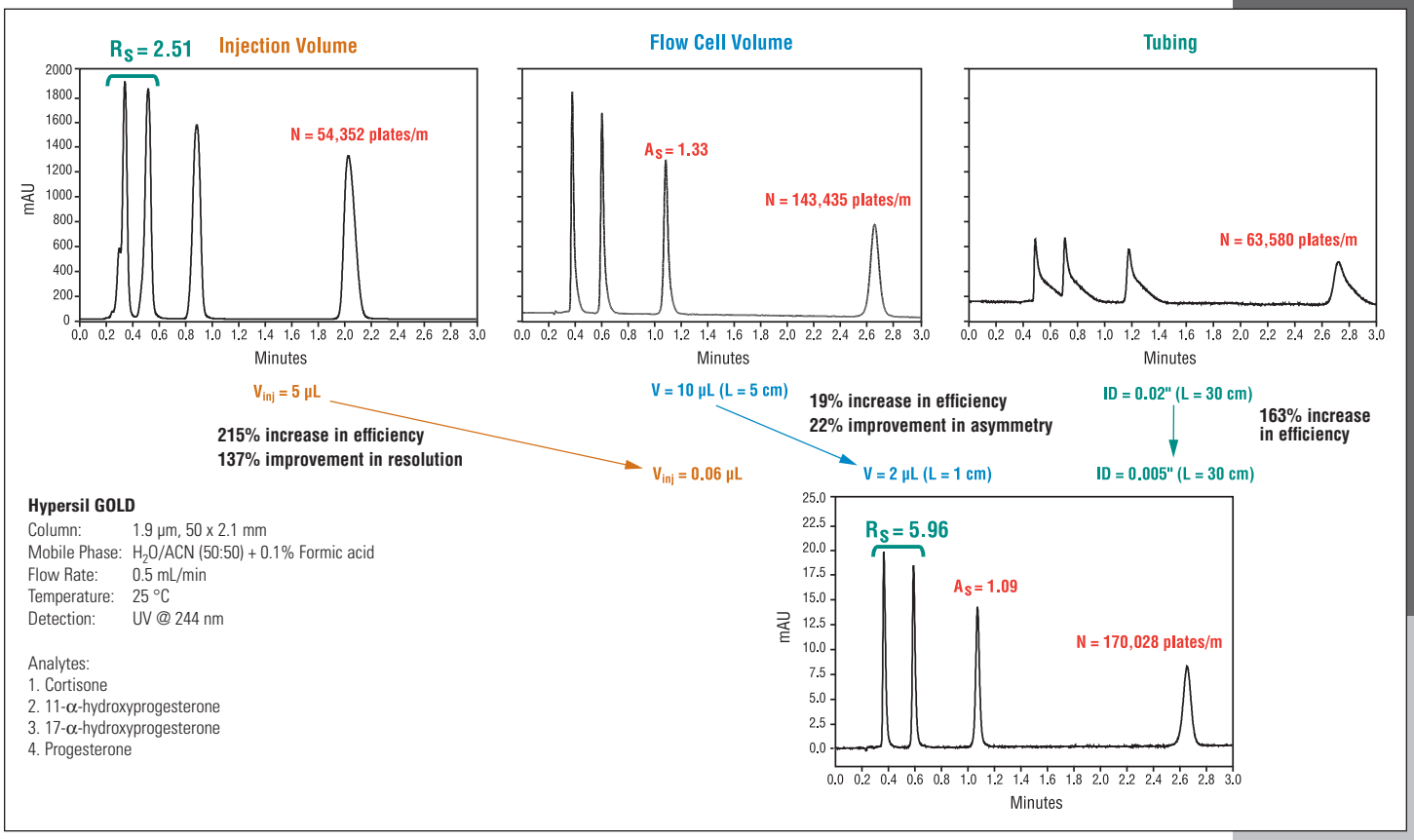
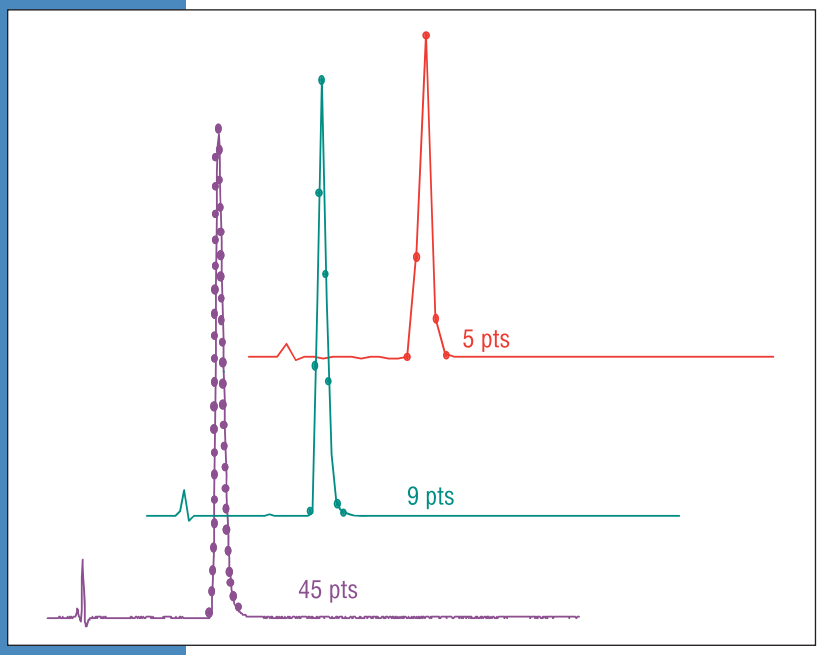


Figure 8: The effects of minimizing volume dispersion within the system.

Detector Sampling Rate

With 1.9 µm particles, operating parameters can be optimized to give fast analysis. This results in narrow chromatographic peaks which may be of the order of 1-2 seconds in width. It is important to scan the detector (whether it is UV or MS) fast enough to achieve optimum peak definition, otherwise resolution, efficiency and analytical accuracy will be compromised. This is illustrated in Figure 9, which illustrates a loss of resolution and peak height when only five scan points cover the peak.



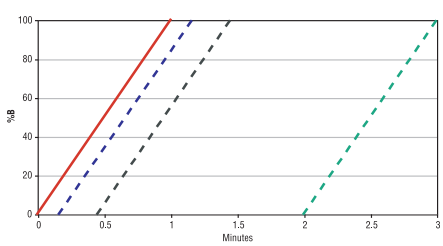
Dwell Volume

The HPLC pump dwell volume is extremely important when running high speed applications using ballistic gradients, typical of high throughput separations on small particle packed columns. This is because the pump dwell volume affects the time it takes for the gradient to reach the head of the column.

If we consider a method using a flow rate of 0.4 mL/min and a fast gradient of 1 minute, the theoretical gradient reaches the column immediately (Figure 10). A pump with a 65 µL dwell volume (such as used in the Thermo Scientific Accela Fast HPLC) will get the gradient onto the column in 9.75 seconds. A traditional quaternary pump with a dwell volume of 800 µL will take 2 minutes to get the gradient to the column. When running rapid gradients this is too slow as can be seen on the example chromatogram.

In Figure 10 A and B the same 2 minute gradient was run on a pump with a 800 µL dwell volume, and a pump with a 80 µL dwell volume. The chromatograms are very different: for chromatogram A, it was necessary to introduce an isocratic hold at the end of the 2 minutes gradient to allow elution of the analytes. In these conditions the pump dwell volume can double the run time, and it also impacts on column re-equilibration at the end of the run.

Figure 9: Effects of detector sampling rate on peak shape. With the use of 1.9 µm particles, it is important that the detector scan rate is fast enough to maintain peak definition. In this figure, there is a dramatic difference in peak shape when only five data points (slower scan rate) are acquired compared to 45 data points (faster scan rate).



- Theoretical gradient reaches the column at 0 seconds
- **65 µL** dwell volume at **0.4 mL/min**, gradient reaches the column at 9.75 seconds
- **180 µL** dwell volume at **0.4 mL/min**, gradient reaches the column at 27 seconds
- **800 µL** dwell volume at **0.4 mL/min**, gradient reaches the column at 120 seconds

Hypersil GOLD

Column:	1.9 µm, 50 x 2.1 mm
Mobile Phase:	A: H_2O + 0.1% Formic acid B: ACN + 0.1% Formic acid
Gradient:	5 to 100% B in 2 min
Flow Rate:	0.55 mL/min
Temperature:	25 °C
Detection:	UV @ 270 nm
Injection Volume:	0.5 µL

Analytes:
1. Sulphaguanidine
2. Sulphamerazine
3. Sulphamonomethoxine
4. Sulphaquinoxaline

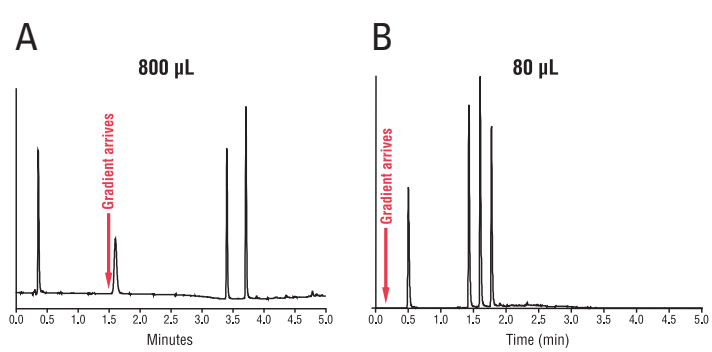


Figure 10: The effects of pump dwell volume on the separation. When running rapid gradients it is important to utilize a pump with a small dwell volume.

System Pressure

In order to tune your assay to your HPLC system, remember that as the particle size is reduced, resistance to flow increases, causing pressure drop to increase within the system. This is approximated by the Equation 9 below:

$$\text{Pressure Drop (psi)} \sim 250\ L\ \eta\ F / d_p^2\ d_c^2 \qquad (9)$$

where L = Column Length (mm)
η = Mobile Phase Viscosity (cP)
F = Flow Rate (mL/min)
d_p = Particle Diameter (µm)
d_c = Column Internal Diameter (mm)

This equation shows that the pressure drop across the column varies with:

- **The length of the column.** Longer columns have higher pressure drops.
- **The ID of the column.** Narrower columns have higher pressure drops.
- **The diameter of the particles packed within the column.** The smaller the particles the higher the pressure drop. This is a squared relationship and has a significant effect.
- **The flow rate.** A higher flow rate will result in a higher pressure drop.
- **The viscosity of the mobile phase.** Higher viscosities will result in higher pressure drops. Increased temperatures reduce the viscosity, enabling a higher flow rate to be used for an equivalent pressure drop.

For the fastest results from small particle columns, you must ensure that your system can function reliably at higher operating pressure. Figure 11 shows that the flow rates for optimum efficiency (taken from the van Deemter plot) can lie within the limits of conventional HPLC systems, even for 1.9 µm particles.

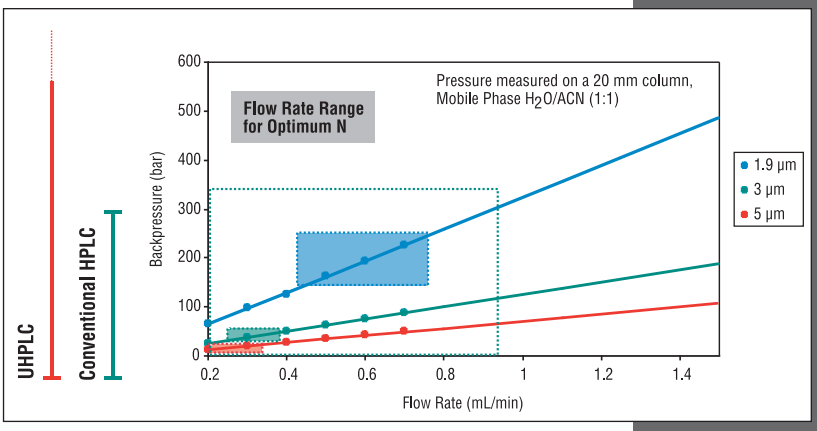
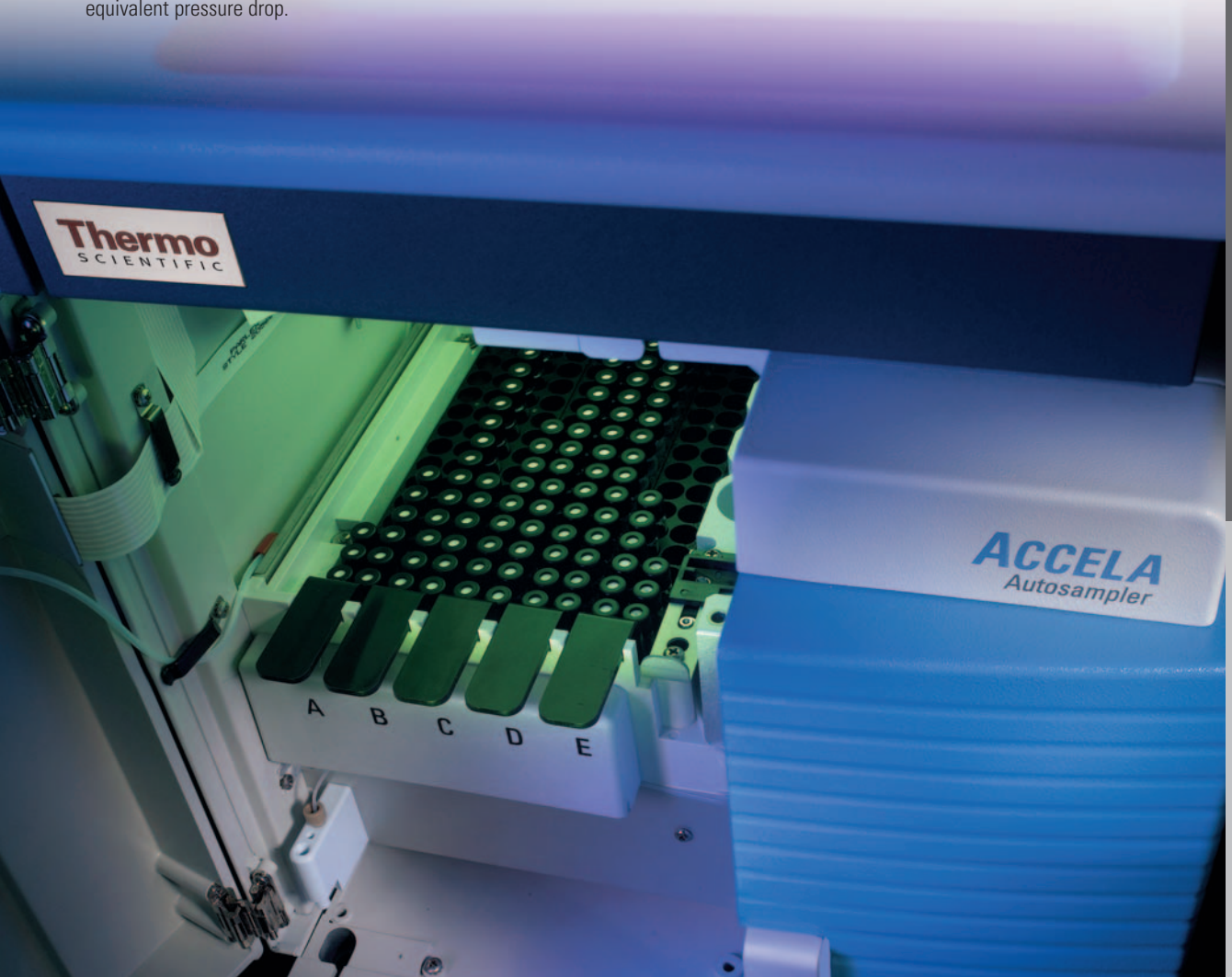


Figure 11: System pressure considerations with 1.9 µm particles. The shaded areas indicate typical system pressures at the optimum flow rates, measured on a 20 mm length column.



Section 3: Tips for Method Transfer

As well as particle size, column dimensions can be scaled down to achieve faster separations. Care must be taken when transferring a method to shorter columns with smaller particles to ensure operating flow rate and gradient profiles are scaled to keep the assay profile consistent. An understanding of some practical calculation routines help to achieve the scaling.

Isocratic Method Transfer

1) Adjust flow rate, keeping linear velocity constant between the original and new method.

System 1	System 2
Column 1: 100 x 4.6 mm, 5 µm	Column 2: 100 x 2.1 mm, 1.9 µm
Flow Rate 1: 1.0 mL/min	Flow Rate 2: 0.55 mL/min
$F_2 = F_1 \times (d_{c2}^2/d_{c1}^2) \times (d_{p1}/d_{p2})$	
F_1 – original flow rate (mL/min)	F_2 – new flow rate (mL/min)
d_{c1} – original column internal diameter (mm)	d_{c2} – new column internal diameter (mm)
d_{p1} – original column particle size (mm)	d_{p2} – new column particle size (mm)

Entering the column dimensions for both systems and the original flow rate results in a calculated flow rate of 0.55 mL/min for system 2. Transferring this method to a column packed with 1.9 µm particles gives a 4.8x increase in sensitivity and 2x increase in speed.

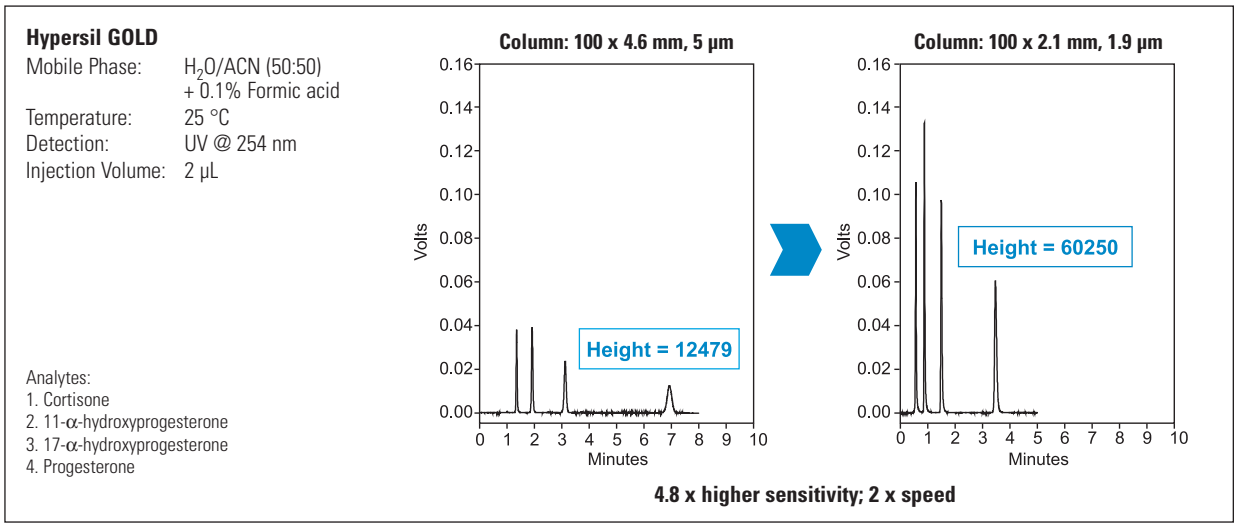


Figure 12: Isocratic method transfer to narrower ID column packed with smaller particles.

2) Adjust injection volume.

System 1	System 2
Column 1: 100 x 4.6 mm	Column 2: 50 x 2.1 mm
V_{i1} = 10 µL	V_{i2} = 1 µL
$V_{i2} = V_{i1} \times (d_{c2}^2 \times L_2/d_{c1}^2 \times L_1)$	
V_{i1} – original injection volume (µL)	V_{i2} – new injection volume (µL)
d_{c1} – original column internal diameter (mm)	d_{c2} – new column internal diameter (mm)
L_1 – original column length (mm)	L_2 – new column length (mm)

Entering the column dimensions for both systems and the original injection volume results in an injection volume of 1 µL for system 2.

In practical terms it is often not possible to follow the equation exactly, due to sample constraints, but the chromatographer should be aware that smaller columns packed with 1.9 µm particles will require a smaller injection volume.

Method Transfer for Increased Resolution

When transferring a method to a column packed with small particles it is possible to optimize certain chromatographic parameters to further increase resolution for difficult separations in complex matrices. In the example below, using the same analytes, the column length was maintained and the flow rate and column ID decreased. This results in a 13% increase in resolution between peaks 1 and 2, as shown in Figure 13.

Method Transfer for Increased Speed

If the analyte peaks are well separated and high throughput is the most important consideration for a method, it is possible to increase the chromatographic speed by further reducing the column length and increasing the flow rate. In Figure 14, the calculated method transfer flow rate comes out at 0.55 mL/min. This and the shorter column used reduces the retention time for peak 4 from 416 seconds to 95 seconds. To obtain an even faster analysis, the separation has been repeated on a shorter column with a higher flow rate, giving a retention time for peak 4 of 29 seconds. This is 14 times faster than the original method and underlines the power of small particles for fast analysis.

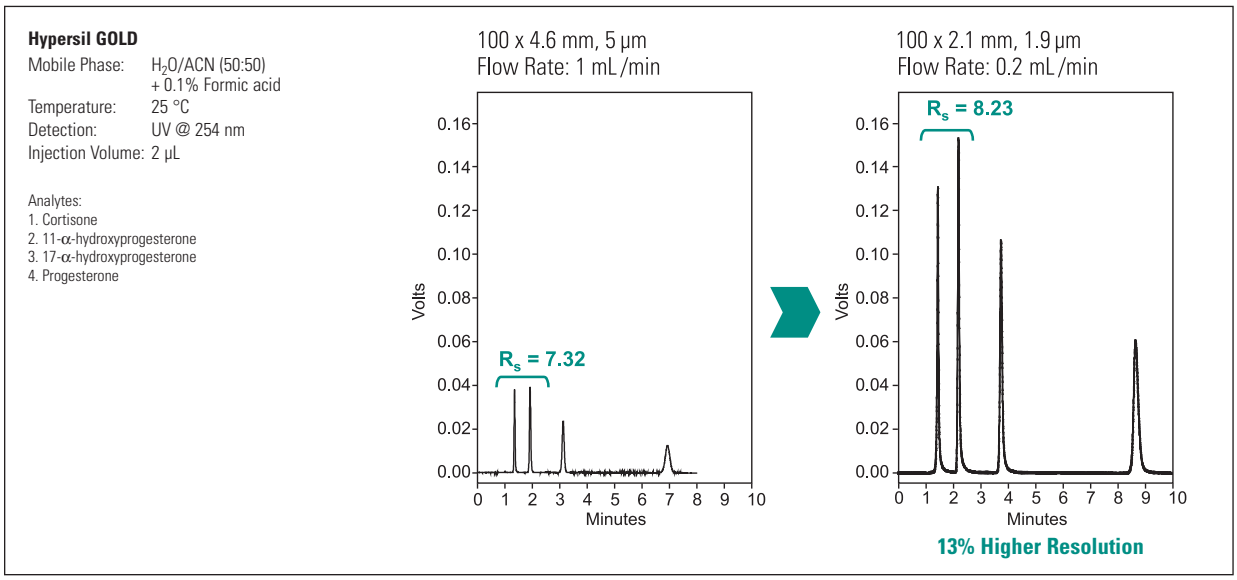


Figure 13: Isocratic method transfer to improve resolution.

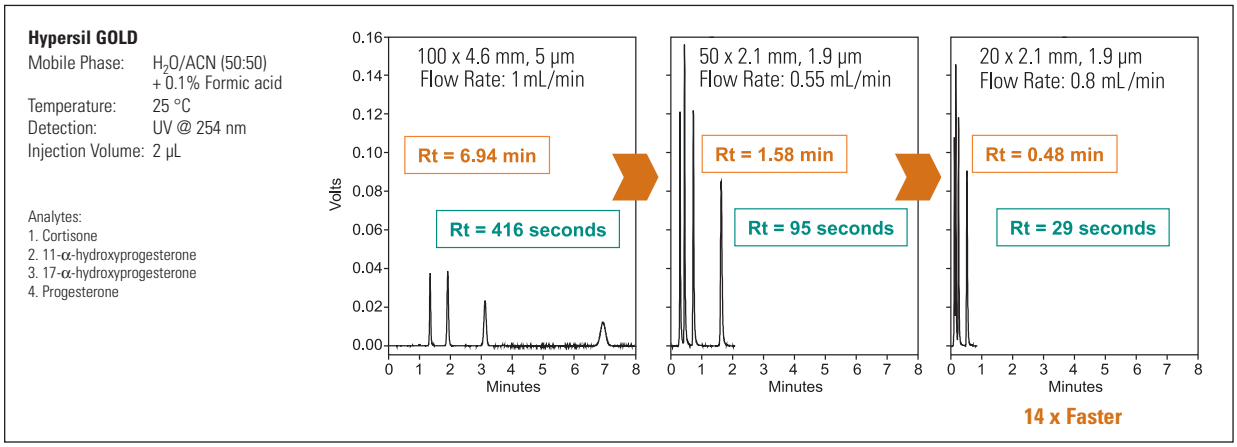


Figure 14: Isocratic method transfer to improve speed. The column has been shortened further and the flow rate increased to 0.8 mL/min.

Gradient Method Transfer

1) Adjust flow rate, keeping linear velocity constant between the original and new method.
(in accordance with the isocratic method transfer equations on page 10)

2) Adjust injection volume. *(in accordance with the isocratic method transfer equations on page 10)*

3) Adjust gradient, keeping initial and final composition unchanged.

System 1	System 2
Column 1: 150 x 4.6 mm	Column 2: 50 x 2.1 mm
Gradient Profile 1 (t_{g1}): 5 to 50% in 15 mins	Gradient Profile 2 (t_{g2}): 5 to 50% in 5 mins
Column Volume: 1.7 mL	Column Volume: 0.11 mL
$t_{g2} = t_{g1} \times (V_{02}/V_{01}) \times (F_1/F_2)$	
t_{g1} – gradient time in original method (min)	t_{g2} – gradient time in new method (min)
V_{01} – original column volume (mL)	V_{02} – new column volume (mL)
F_1 – original flow rate (mL/min)	F_2 – new flow rate (mL/min)

Entering the column dimensions and flow rates for both systems and the original gradient gives a t_g of 5 minutes for system 2.

Column Volume (V_0)
$V_0 = 0.68 \times \pi \times r^2 \times L$
V_0 – column void volume (mL)
L – column length (cm)
r – column radius (cm)
0.68 is the approximate fraction of the column volume occupied by mobile phase (for porous particles)

In the example chromatograms on the following page (Figure 15), a gradient method for separating sulphonamides has been transferred from a standard 5 µm analytical column to a shorter column containing 1.9 µm particles. This has been performed stepwise to illustrate the effect of each parameter change. For each step the gradient and flow rate have been scaled in accordance with the previous calculations. The final chromatogram illustrates where the column has been further shortened and the flow rate and gradient made even faster to increase throughput.

The table compares key parameters from the start and end methods and shows that even under ultra fast conditions (the final gradient is 15 times faster than the starting gradient), baseline separation is still achieved. The peak heights are similar even though the injection volume is 10% of the starting injection volume accentuating the increased sensitivity achieved using 1.9 µm particles.

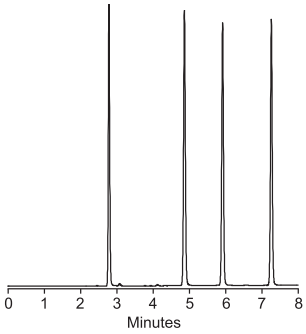
Hypersil GOLD

Column: 150 x 4.6 mm, 5 µm **(V = 1.7 mL)**
Gradient: 5 to 100% B in **15 minutes**
Mobile Phase: A: H_2O + 0.1% Formic acid
B: ACN + 0.1% Formic acid
Temperature: 25 °C
Detection: UV @ 254 nm
Injection Volume: **5 µL**
Flow Rate: **1 mL/min**

Analytes:
1. Sulphaguanidine
2. Sulphamerazine
3. Sulphamonomethoxine
4. Sulphaquinoxaline

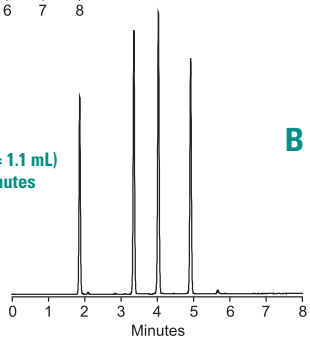
	150 x 4.6 mm, 5 µm Chromatogram A	30 x 2.1 mm, 1.9 µm Chromatogram E
Peak Width* (s)	9.0	4.2
	13.2	3.6
	10.2	3.0
	11.4	2.4
Resolution*	11.2	8.8
	5.4	2.0
	7.4	2.2
Peak Height	236331	71764
	231151	139283
	220165	170483
	222985	135603

** baseline*



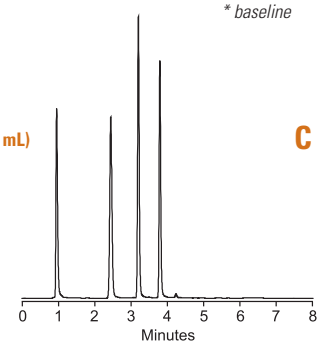
1

Column: 100 x 4.6 mm, 5 µm **(V = 1.1 mL)**
Gradient: 5 to 100% B in **10 minutes**
Flow Rate: **1 mL/min**



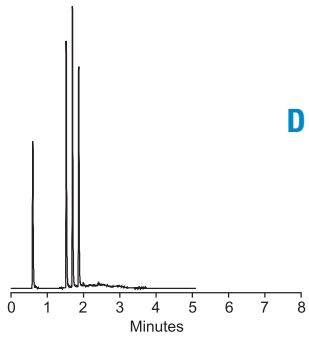
2

Column: 50 x 2.1 mm, 5 µm **(V = 0.11 mL)**
Gradient: 5 to 100% B in **5 minutes**
Flow Rate: **0.2 mL/min**



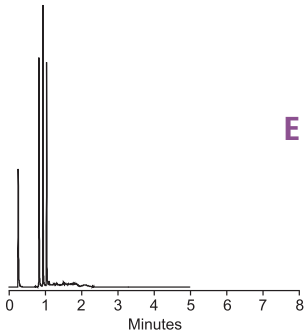
3

Column: 50 x 2.1 mm, 1.9 µm **(V = 0.11 mL)**
Gradient: 5 to 100% B in **2 minutes**
Flow Rate: **0.55 mL/min**
Injection Volume: **0.5 µL**



4

Column: 30 x 2.1 mm, 1.9 µm **(V = 0.07 mL)**
Gradient: 5 to 100% B in **1 minutes**
Flow Rate: **0.7 mL/min**
Injection Volume: **0.5 µL**



Cycle Time Reduced by 10 Fold

Figure 15: Stepwise transfer of a gradient method to 1.9 µm Hypersil GOLD. In each step, the gradient and flow rate are scaled using the calculations presented. In step 1, column length was reduced. Step 2 shows the effects of a reduction in column ID. In Step 3 particle size is reduced to 1.9 µm. In Step 4, the column length is decreased again, while the flow rate is increased.

Section 4: Column Reproducibility

Hypersil GOLD columns are exceptionally reproducible for reliable chromatography, column after column. This allows the user to be confident that assays developed with Hypersil GOLD columns will be robust and stable for the life of the assay, making them the ideal choice for new method development.

Built on 30 years of experience in product development and manufacturing of HPLC media and columns, Thermo Scientific Hypersil GOLD consumables continue upon their success with the development of a new state-of-the-art family of columns, based on 1.9 µm particles, designed for improved chromatography. Hypersil GOLD columns are manufactured in ISO 9001:2000 accredited laboratories under strict protocols using a robust manufacturing procedure and extensive quality control testing.

To obtain the most accurate results when performing quantitative analysis it is important that key chromatographic parameters such as retention time and peak area remain consistent.

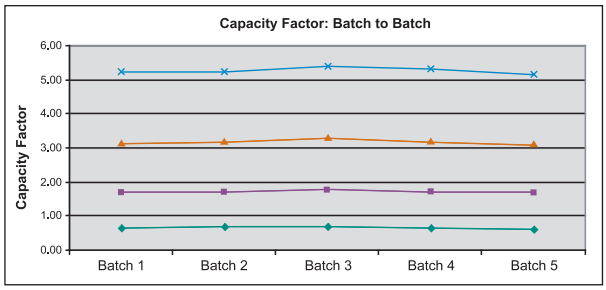


Figure 16: 1.9 µm Hypersil GOLD Batch Reproducibility. The capacity factor was measured for a series of peaks across 5 separate batches of 1.9 µm Hypersil GOLD. The reproducible values provide the chromatographer with confidence that analyte peaks will elute at the same time, every time.

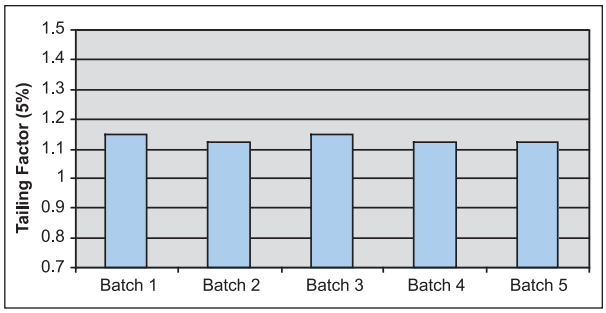


Figure 18: Batch Reproducibility with 1.9 µm Hypersil GOLD Columns. For all batches, the tailing factor measured for a very basic analyte, is close to unity, highlighting the excellent symmetrical peak shape which is characteristic of all Hypersil GOLD columns.

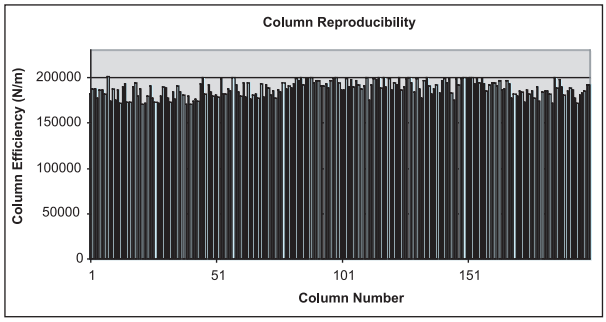


Figure 17: 1.9 µm Hypersil GOLD Column Reproducibility. 1.9 µm Hypersil GOLD columns show excellent reproducibility, column after column. In this example, the efficiency of 200 different columns is shown. Efficiency in excess of 160,000 plates/m is consistently achieved, column after column.

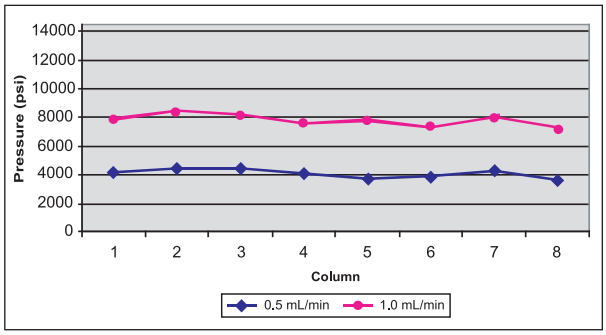


Figure 19: System Back Pressure with 1.9 µm Hypersil GOLD. The system back pressure has been measured over eight separate columns at two flow rates. The minimal variation in column back pressure can only be achieved using consistently well-packed stationary phase with a reproducible narrow particle size distribution.

Assay Reproducibility

The analysis of a mixture of sulphonamides using 1.9 µm Hypersil GOLD column gives a linear response over a range of concentrations between 25 and 250 ng/mL (Figure 20).

The table shows excellent reproducibility for retention times (< 1% RSD) and peak area (< 2% RSD) over six injections. This highlights that accurate data can be obtained under fast chromatographic conditions, where peak widths might be as narrow as 1-2 seconds.

Hypersil GOLD, 1.9 µm, 50 x 2.1 mm

Mobile Phase: A: H_2O + 0.1% Formic acid
B: ACN + 0.1% Formic acid
Gradient: 5 to 100% B in 2 min
Flow Rate: 0.55 mL/min
Temperature: 25 °C
Detection: UV @ 270 nm
Injection Volume: 0.5 µL

Analytes:
1. Sulphaguanidine
2. Sulphamerazine
3. Sulphamonomethoxine
4. Sulphaquinoxaline

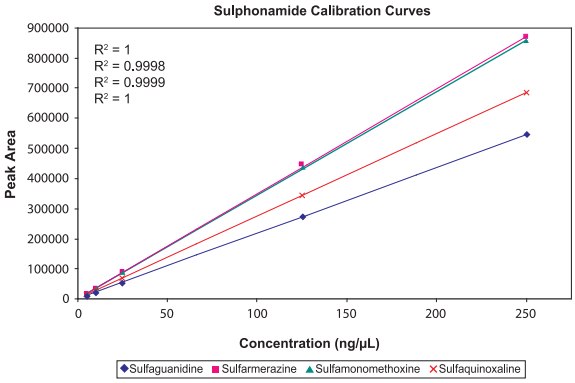


Figure 20: Calibration Curves for Sulphonamides. 1.9 µm Hypersil GOLD columns produce a linear response over a range of concentrations.

	Rt (min) Mean	Rt (min) STD	Rt (min) RSD (%)	Peak Area Mean	Peak Area STD	Peak Area RSD (%)
Sulphaguanidine						
Level 1 (25 ng/µL)	0.50	0.000	0.00	58066	1153	1.99
Level 2 (50 ng/µL)	0.51	0.005	0.93	109285	920	0.84
Level 3 (250 ng/µL)	0.51	0.004	0.73	545729	2962	0.54
Sulphamerazine						
Level 1 (25 ng/µL)	1.06	0.005	0.47	76352	941	1.23
Level 2 (50 ng/µL)	1.10	0.011	0.97	153414	2229	1.45
Level 3 (250 ng/µL)	1.08	0.009	0.83	873540	11653	1.33
Sulphamonomethoxine						
Level 1 (25 ng/µL)	1.19	0.005	0.42	107601	1389	1.29
Level 2 (50 ng/µL)	1.23	0.010	0.78	182809	2954	1.62
Level 3 (250 ng/µL)	1.22	0.011	0.83	859790	14827	1.72
Sulphaquinoxaline						
Level 1 (25 ng/µL)	1.32	0.004	0.28	74714	1252	1.68
Level 2 (50 ng/µL)	1.36	0.012	0.85	146760	2225	1.52
Level 3 (250 ng/µL)	1.35	0.007	0.51	688423	10197	1.48

Ordering Information

Description	Particle Size	Length (mm)	3.0 mm ID	2.1 mm ID	1.0 mm ID	320 µm ID
Hypersil GOLD	1.9 µm	20	25002-023030	25002-022130	–	–
		30	25002-033030	25002-032130	25002-031030	–
		50	25002-053030	25002-052130	25002-051030	25002-050365
		100	25002-103030	25002-102130	25002-101030	25002-100365
		200	–	25002-202130	–	–
Hypersil GOLD aQ	1.9 µm	20	25302-023030	25302-022130	–	–
		30	25302-033030	25302-032130	25302-031030	–
		50	25302-053030	25302-052130	25302-051030	25302-050365
		100	25302-103030	25302-102130	25302-101030	25302-100365
		200	–	25302-202130	–	–
Hypersil GOLD PFP	1.9 µm	20	25402-023030	25402-022130	–	–
		30	25402-033030	25402-032130	25402-031030	–
		50	25402-053030	25402-052130	25402-051030	25402-050365
		100	25402-103030	25402-102130	25402-101030	25402-100365
		200	–	25402-202130	–	–

Other custom column dimensions are available. Please call your local Customer Service for more information.

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