Achieving Ultra-High Performance

5678

Guide to Scaling with Kinetex® Columns



Innovation in Particle Technology

The Kinetex[®] core-shell particle is not fully porous. Using sol-gel processing techniques that incorporate nano structuring technology, a durable, homogenous porous shell is grown on a solid silica core. This highly optimized process combined with uniform particle size distribution

produces a column that generates extremely high plate counts on par with sub-2 μ m particles. When using Kinetex 2.6 μ m core-shell columns, less column backpressure is generated, allowing it to be used on any LC system.**



Kinetex 1.7 µm Core-Shell Particle

- Reduced controlled diffusion path maximizes efficiency
- Increased efficiencies compared to traditional fully porous sub-2 µm columns. Typical operating backpressures > 400 bar



Traditional Fully Porous Particle

- Long and variable diffusion path limits efficiencies
- Ultra-high performance limited to UHPLC systems with traditional fully porous sub-2 µm columns



** When using Kinetex 1.7 µm, increased performance can be achieved, however higher pressure-capable instrumentation is required.



Increase Efficiency with Kinetex® Core-Shell Technology

Column efficiency (N), the measure of theoretical plates over a given length, is one of the most important indicators of column performance. Efficiency is heavily influenced by:

- Column length
- Particle size

This relationship can be illustrated with the following equation:



= Reduced Plate Height (Typically 2-2.5, assume 2.2 for calculations)

= Particle Diameter (mm)

Using this equation, a column's theoretical efficiency may be estimated.

Example:

- A 150 x 4.6 mm 5 μm column would have its efficiency calculated as follows:

$$N = \frac{150}{(2.2).005} \longrightarrow N = \frac{150}{.011} \longrightarrow N = 13,636$$
 plates

• Then, to convert to plates/ meter

 $N_{p/m} = [13636 \text{ plates / length of column (mm)}] \times 1000 \text{ mm/m}$ $\rightarrow N_{p/m} = (13636 / 150 \text{ mm}) \times 1000 \text{ mm/m} = 90,906 \text{ p/m}$

Smaller particle size columns will produce higher efficiencies.





Increase Efficiency with Kinetex® Core-Shell Technology

In order to more accurately predict the efficiency of Kinetex columns using the efficiency equation, an effective particle size d_e of 1.7 μ m must be used (Figure 1). As such:

An effective d_p of 1.7 μm is used for Kinetex 2.6 μm particles An effective d_p of 1.5 μm is used for Kinetex 1.7 μm particles

Figure 1. van Deemter plot of Fully Porous 3 and 1.7 μm Column, and the Kinetex 2.6 μm Core-Shell Column



What would be the calculated change in Efficiency from...





Approximately **2x** Increase in Efficiency

Increase Resolution

What is resolution?

Resolution (Rs) describes the separation power of the complete chromatographic system relative to the components of the mixture. Through obtaining optimal resolution, scientists are able to:

- 1. Identify more compounds
- 2. Decrease run times
- 3. Increase solvent savings

$$R_{s} = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k}{k+1} \right)$$

Resolution is proportional to the square root of N (the column efficiency)

Compounds are baseline resolved when resolution is ≥ 1.5 Compounds are not baseline resolved when resolution is < 1.5.

It is clear that large increases in efficiency can significantly increase resolution.

Example:

If column efficiency triples, by going from a 5 µm column to a Kinetex[®] 2.6 µm column of equivalent dimensions (see pg. 5), what would be the expected impact to resolution?

With a 3x increase in efficiency ($\Delta N=3$)

Resolution will increase by $\sqrt{3}$ \rightarrow 73 % increase in Resolution



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Increase Resolution EP Method for Atenolol and Related Impurities



* Waters and Symmetry are registered trademarks of Waters Corporation. Phenomenex is in no way affiliated with Waters Corporation. Comparative separations may not be representative of all applications.

Increase Resolution

Kinetex[®] columns are the highest efficiency columns sold today. Selecting an equivalent length Kinetex column and optimizing flow for the particle will provide the highest R_s .

Isocratic Separations

A. Scale the flow rate to achieve the same mobile phase linear velocity with the new column ID.

Flow Rate_{Kinetex} = Flow Rate_{Original}
$$x \left(\frac{\text{diameter}_{Kinetex}}{\text{diameter}_{Original}} \right)$$

Example: 1 mL/min flow rate 4.6 mm ID to 3.0 mm ID

1 mL/ min x
$$\left(\frac{3.0 \text{ mm}}{4.6 \text{ mm}}\right)^2$$
 = 0.43 mL/min

Note: considerations should be made for system backpressure & flow rate limitations.

B. Scale the injection volume to account for change in column ID.

$$Inj.vol._{Kinetex} = Inj.vol._{Original} \times \left(\frac{diameter_{Kinetex}}{diameter_{Original}}\right)^{2}$$





The mobile phase linear velocity may be adjusted in line with the reduced effective particle size.

Flow Rate_{Kinetex} $x \left(\frac{d_{p \text{ Original}}}{d_{e \text{ Kinetex}}} \right)$

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optional

Increase Resolution

Gradient Separations

A. Scale the flow rate to achieve the same mobile phase linear velocity with the new column ID.

Flow Rate_{Kinetex} = Flow Rate_{Original}
$$x \left(\frac{\text{diameter}_{\text{Kinetex}}}{\text{diameter}_{\text{Original}}} \right)^2$$

B. Scale the injection volume to account for change in column ID.

$$\textit{Inj.vol.}_{\textit{Kinetex}} = \textit{Inj.vol.}_{\textit{Original}} x \left(\frac{\textit{diameter}_{\textit{Kinetex}}}{\textit{diameter}_{\textit{Original}}}\right)^{2}$$

C. To match your original gradient program, adjust the time segment at each step to maintain the same column volume (cv) per unit time. The calculated time segment will take into account changes in column ID, flow rate, and column length.

$$Time \ Segment_{Kinetex} = Time \ Segment_{Original} x \left(\frac{ID_{Kinetex}}{ID_{Original}}\right)^2 x \left(\frac{Flow \ Rate_{Original}}{Flow \ Rate_{Kinetex}}\right) x \left(\frac{Column \ Length_{Kinetex}}{Column \ Length_{Original}}\right)$$

The mobile phase linear velocity may be adjusted in line with the reduced effective particle size.

Flow Rate_{Kinetex}
$$x \left(\frac{d_{p \text{ original}}}{d_{e \text{ Kinetex}}} \right)$$

optional

Increase Productivity

Productivity may be defined as: unit resolution per unit time

Resolution must be maintained as run time is reduced or no improvement in productivity is realized. To choose the correct length Kinetex[®] column for increasing productivity, we recommend using a simple ratio comparison:



A comparison of these ratios will be indicative of the difference in resolution power between the two columns. A shorter Kinetex column, providing the resolving power of the original column, will maintain resolution while decreasing run time.

Comparison Examples







Increase Productivity

Isocratic Separations

A. Choose the correct Kinetex[®] column:

Column Length_{Original} : Column Length_{Kinetex} d_{p Original} : d_{e Kinetex}

B. Scale the flow rate to achieve the same mobile phase linear velocity with a new column ID. Flow Rate_{Kinetex} =Flow Rate_{Original} $x \left(\frac{diameter_{Kinetex}}{diameter_{Original}} \right)$

The mobile phase linear velocity may be adjusted in line with the reduced effective particle size.

100 90 80 70 80 100 10 120 130 80

Flow Rate_{Kinetex} $x\left(\frac{d_{POriginal}}{d_{eKinetex}}\right)$

optional

Gradient Separations

 $\frac{\text{Column Length}_{\text{Original}}}{d_{p \text{ Original}}} : \frac{\text{Column Length}_{\text{Kinetex}}}{d_{e \text{ Kinetex}}}$

- B. Scale the flow rate to achieve the same mobile phase linear velocity with a new column ID. Flow Rate_{Kinetex} =Flow Rate_{Original} $x \left(\frac{diameter_{Kinetex}}{diameter_{Original}}\right)^2$
- C. To match your original gradient program, adjust the time segment at each step to account for any changes in flow rate, ID, and column length:

$$Time \ Segment_{Kinetex} = Time \ Segment_{Original} x \left(\frac{Column \ Length_{Kinetex}}{Column \ Length_{Original}}\right) x \left(\frac{Flow \ Rate_{Original}}{Flow \ Rate_{Kinetex}}\right) x \left(\frac{ID_{Kinetex}}{ID_{Original}}\right)^2$$

optional

Flow Rate_{Kinetex} $x \left(\frac{d_{p \text{ Original}}}{d_{e \text{ Kinetex}}} \right)$

Increase Throughput

Optimum throughput is achieved by decreasing total analysis time as much as possible while maintaining acceptable chromatographic performance ($R_s \ge 1.5$).

Conventional way to increase throughput



Kinetex[®] way to increase throughput



Increase Throughput

 $R_s 5, 6 = 3.4$

2

36

16x Increase in Throughput

25x Increase in Throughput!



Columns:	Fully Porous	s 5 µm C18	3(2)		Sample:	1.	Pyridine
Dimensions:	250 x 4.6 m	250 x 4.6 mm				2.	Acetaminophen
Mobile Phase:	A: 0.1 % Fo	A: 0.1 % Formic acid / Water				3.	Quinine
	B: 0.1 % Fo	rmic acid /	Aceton	itrile		4.	Acebutolol
Gradient:	Step No.	Time	% A	% B		5.	Chlorpheniramine
	1.	0	95	5		6.	Triprolidine
	2.	2.79	95	5		7.	Prednisolone
	3.	36.15	5	95		8.	4-Chlorobenzoic acid
	4.	36.38	95	5		9.	4-Chlorocinnamic acid
	5.	50.93	95	5		10.	Diazepam
Flow Rate:	1 mL/min					11.	Diflunisal
Temperature:	45 °C					12.	Hexanophenone
Detection:	UV @ 254 n	m (25 °C)					
Backpressure:	106 bar						

Columns: Dimensions: Part No.: Mobile Phase:	Kinetex® 2.6 µ 50 x 4.6 mm 00B-4462-E0 A: 0.1 % Form	im C18 ic acid /	/ Water	itrilo	Sample:	1. 2. 3. 4.	Pyridine Acetaminophen Quinine Acebutolol
Gradient:	B: 0.1 % Form Step No. 1. 2. 3. 4.	Time 0 0.20 2.47 2.48 3.47	/ Aceton % A 95 95 5 95 95	1trile % B 5 5 95 5 5		5. 6. 7. 8. 9. 10.	Chlorpheniramine Triprolidine Prednisolone 4-Chlorobenzoic acid 4-Chlorocinnamic acid Diazepam Diflunisal
Flow Rate: Temperature: Detection: Backpressure:	2.94 mL/min 45 °C UV @ 254 nm 300 bar	(25 °C)		Ū		12.	Hexanophenone

Columns:	Kinetex 2.6 µr	inetex 2.6 µm C18				1.	Pyridine
Dimensions:	50 x 4.6 mm				-	2.	Acetaminophen
Part No.:	00B-4462-E0	00B-4462-E0				3.	Quinine
Mobile Phase:	A: 0.1 % Form	ic acid	/Water			4.	Acebutolol
	B: 0.1 % Form	ic acid	/ Acetoni	trile		5.	Chlorpheniramine
Gradient:	Step No.	Time	% A	% B		6.	Triprolidine
	1.	0	95	5		7.	Prednisolone
	2.	0.14	95	5		8.	4-Chlorobenzoic acid
	3.	1.59	5	95		9.	4-Chlorocinnamic acid
	4.	1.60	95	5		10.	Diazepam
	5.	2.23	95	5		11.	Diflunisal
Flow Rate:	4.6 mL/min					12.	Hexanophenone
Temperature:	45 °C						
Detection:	UV @ 254 nm (25 °C)						
Backpressure:	485 bar						

12

9 | 10 ¹¹

7 8

Choosing the Best Kinetex® Column

Expected Backpressure at Different Flow Rates*

There is an optimal Kinetex column for your system and operating conditions. Use these graphs to determine the starting Kinetex particle size and dimension for your method.

50:50 (Acetonitrile/Water)



60:40 (Methanol/Water)



Material Characteristics

Packing Material	Total Particle Size (µm)	Porous Shell (µm)	Solid Core (µm)	Pore Size (Å)	Effective Surface Area (m²/g)	Effective Carbon Load %	pH Stability	Pressure Stability
Kinetex C18	2.6	0.35	1.9	100	200	12	1.5-10	
Kinetex XB-C18	2.6	0.35	1.9	100	200	10	1.5-10	
Kinetex C8	2.6	0.35	1.9	100	200	8	1.5-10	600 bar
Kinetex PFP	2.6	0.35	1.9	100	200	9	1.5-8.0	
Kinetex HILIC	2.6	0.35	1.9	100	200	0	2.0-7.5	
Kinetex C18	1.7	0.23	1.25	100	200	12	1.5-10	
Kinetex XB-C18	1.7	0.23	1.25	100	200	10	1.5-10	
Kinetex C8	1.7	0.23	1.25	100	200	8	1.5-10	1000 bar
Kinetex PFP	1.7	0.23	1.25	100	200	9	1.5-8.0	
Kinetex HILIC	1.7	0.23	1.25	100	200	0	2.0-7.5	

*Due to variation in system, sample and method parameters, graphs provided may not be representative of all applications. Data generated on Agilent 1200 SL.

KINETEX CALCULATOR!

Instantly optimize your method at *www.phenomenex.com/optimize* OR contact Your Phenomenex representative for optimization assistance.



Ordering Information

Kinetex[®] 2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6
XB-C18		00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0
C8	_	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0
HILIC	_	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0

Kinetex 2.6 µm MidBore™ Columns (mm)

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0
XB-C18		00B-4496-Y0		00D-4496-Y0	
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0
C8		00B-4497-Y0	_	00D-4497-Y0	
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0
HILIC					00F-4461-Y0

Kinetex 2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1
XB-C18		00B-4496-AN	00D-4496-AN	
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN
C8		00B-4497-AN	00D-4497-AN	
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN
HILIC		00B-4461-AN	00D-4461-AN	00F-4461-AN

Kinetex 1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1
XB-C18	00B-4498-AN	00D-4498-AN	—
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN
C8	00B-4499-AN	00D-4499-AN	_
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN
HILIC	00B-4474-AN	_	_

*SecurityGuard Ultra cartridges require holder, Part No.: AJ0-9000. Check for availability in your country.



Phenex[™] RC (Regenerated Cellulose) Syringe Filters

- Rapid filtration of HPLC and GC samples prior to analysis
- Particulate, PVC, and extractable-free filters
- Universal filter compatible with both aqueous and mixed organic solutions

Choose filter diameter based on sample volume



	4 mn for ≤ 2 mL	sample volu	mes	15 mr for 2 - 10 m	n Diameter L sample vol	umes	25 - 28 for 10 - 100 r	mm Diame nL sample vo	lumes	Footnotes:
Membrane Type/Size	Part No.	Unit	Price	Part No.	Unit	Price	Part No.	Unit	Price	 26 mm diameter. Additional dimensions and membrane
0.20 µm (non-ste	erile)									types are available. Please contact
Phenex-RC	AF0-3203-12	100/ pk		AF0-2203-12	100/ pk		AF0-8203-121	100/ pk		consultant or distributor for availability
(Regenerated Cellulose)	AF0-3203-52	500/ pk		AF0-2203-52	500/ pk		AF0-8203-521	500/ pk		or assistance. 3. Larger quantity purchases at significant savings are available.
0.20 µm (sterile)										
Phenex-RC (Regenerated Cellulose)		_		_			AF0-8459	50/ pk		Note: AF0-8459 is 25 mm diameter.

KrudKatcher[™] Ultra In-line Filter

- Disposable in-line filter fits virtually all UHPLC / HPLC columns 1.0 to 4.6 mm
- Extremely low dead-volume minimizes sample peak dispersion
- Pressure rated to 1375 bar (20,000 psi) (see p. 15 for more information)

Part No.	Description	Unit Price	
F0-8497	KrudKatcher Ultra In-Line Filter, 0.5 µm Porosity x 0.004 in. ID	3/pk	
rudKatcher	Ultra requires 5/16 in. wrench. Installation wrench not provided.		6

UHPLC / HPLC Sure-Lok[™] High Pressure PEEK[®] Male Nut Fittings

UHPLC / HPLC Sure-Lok High Pressure PEEK male nut fittings are recommended for installation of Kinetex columns. The convenient one-piece design (AQ0-8503) is pressure rated to 12,000 psi (827 bar). A handy fitting tightening tool (AQ0-8530) is available to facilitate achievement of a

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Part No.	Description	Unit Price	
AQ0-8503	Sure-Lok High Pressure PEEK 1-Pc Nut, 10-32, for 1/16 in. Tubing,	10/pk	A00-8502
	12,000 psi (827 bar)		AQ0-0505
AQ0-8530	Sure-Lok Fitting Tightening Tool, Aluminum	ea	A00-8530
Sure-Lok Fittin	g Tightening Tool is required for AQ0-8503		A00 0000

3 batch method validation kits available upon request



If you are not completely satisfied with Kinetex[®] core-shell columns, send in your comparative data to a similar product within 45 days and KEEP THE COLUMN FOR FREE.

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