# TN-1063 APPLICATIONS

### Extend Kinetex<sup>™</sup> Core-Shell Column Lifetime with KrudKatcher<sup>™</sup> Ultra In-Line Filters

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The Kinetex core-shell particle technology produces a nearly monodisperse particle size distribution with a significantly shorter diffusion path resulting in significant chromatographic performance benefits (higher column efficiencies and increased resolution) for separation scientists. However, in order to maintain this improved chromatographic performance over time, it is imperative to take precautions to protect Kinetex (and all HPLC/UHPLC) columns from potential contaminants and particulates that might be present in the samples or mobile phase.

### Introduction

For many years, the use of guard columns have been advocated by column manufacturers and other experts to protect and extend column lifetime and performance of analytical columns from potential damage caused by the presence of chemical contaminants and microparticulates in the sample and mobile phase. The introduction and adoption of smaller particle size (< 3  $\mu$ m) and narrow ID columns (≤ 2 mm) columns for ultrahigh performance presents additional challenges. Specifically, these ultra-high performance columns are very sensitive even to small increases in dead volume, and the use of traditional guard columns or guard cartridge systems will show a marked decrease in column performance (efficiency and resolution). However, small particle columns are even more susceptible to contamination and blockage from microparticulates that may be present in both the sample and mobile phases as they can occlude the smaller porosity column frits and dramatically shorten column lifetime.

### **Results and Discussion**

KrudKatcher Ultra in-line filters are specifically designed for use with ultra-high performance columns from 1.0 to 4.6 mm ID and are pressure rated to 20,000 psi (1,375 bar). The filter body houses an integrated 0.5  $\mu$ m 316 stainless steel filter element that efficiently removes microparticulates from the flow stream without contributing to system backpressure or dead volume (<0.2  $\mu$ L). Two separations were run, one isocratic and one gradient, to illustrate the minimal dead volume contribution by the KrudKatcher Ultra in-line filters.

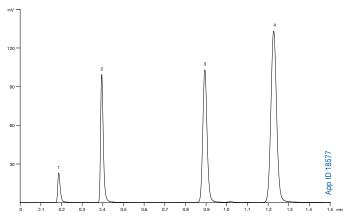
The isocratic separations (Figure 1 & 2) were performed using a simple QC column test mixture. Since isocratic separations will readily highlight increases in system dead volume by changes in retention time and peak width, this is a good measure for determining the contribution from added components such as in-line filters and guard columns to the flow path. The separation conditions are as indicated.

Column:	Kinetex 2.6 µm C18		
	with and without KrudKatcher Ultra In	-Line filte	r as noted
Dimension:	50 x 2.1 mm		
Part No.:	00B-4462-AN (Kinetex column)		
	AF0-8497 (KrudKatcher Ultra In-Line	Filter)	
Mobile Phase:	Acetonitrile / Water (65:35)		
Flow Rate:	0.5 mL/min	Sample:	Reversed Phase QC
Temperature:	22 °C		Test Mix (Part No. AL0-3045)
<b>Injection Volume:</b>	0.2 μL		1. Uracil
Detection:	UV @ 254 nm		2. Acetophenone
			3. Toluene
			4. Naphthalene

### Figure 1.

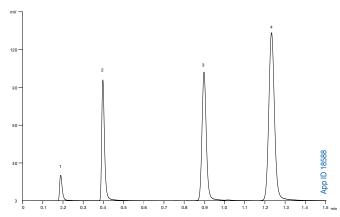
QC test mixture using Kinetex 2.6  $\mu m$  C18 (50 x 2.1 mm) without KrudKatcher Ultra in-line filter

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### Figure 2.

QC test mixture using Kinetex 2.6  $\mu m$  C18 (50 x 2.1 mm) with KrudKatcher Ultra in-line filter





	<i>Without</i> KrudKatcher Ultra In-Line Filter			<b>With</b> KrudKatcher Ultra In-Line Filter		
Analytes	RT (min)	Width (min)	R <sub>s</sub>	RT (min)	Width (min)	$R_{s}$
Uracil	0.188	0.0123		0.190	0.0130	
Acetophenone	0.395	0.0133	9.53	0.399	0.0136	9.21
Toluene	0.894	0.0212	16.94	0.901	0.0215	16.75
Naphthalene	1.227	0.0285	7.87	1.236	0.0288	7.82

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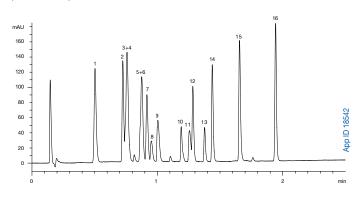
As can be seen from the retention time and peak width data shown in **Table 1**, retention times increased by less than 1% and peak widths increased less than 2.5% as a result of introducing the KrudKatcher<sup>™</sup> Ultra in-line filter. **Table 1** also shows that there were no detrimental effects on chromatographic resolution.

The gradient separations (Figure 3 & 4) were performed using a sixteen component test mixture. The test mixture is quite complex, resulting in some closely eluting chromatographic peaks. In such a separation, any significant increases in system dead volume would contribute to broader peaks and loss of chromatographic resolution. However, comparing the chromatograms obtained with and without KrudKatcher Ultra clearly shows that chromatographic resolution is not compromised, even for the very closely eluting compounds in this sample.

Column:	Kinetex 2.6 μm C18 with and without KrudKatcher Ultra In-Line filter as noted					
Dimension:	50 x 2.1 mm					
Part No.:	00B-4462-AN	(Kinetex c	olumn)			
	AF0-8497 (Kru	dKatcher	Ultra In-Line Fil	ter)		
Mobile Phase:	A: 0.1 % Formi	ic acid in \	Nater			
	B: 0.1 % Form	ic acid in A	Acetonitrile			
Gradient:	Time (min)	% B	Time (min)	% B		
	0	5	2.5	80		
	1.75	80	2.51	5		
Flow Rate:	0.8 mL/min					
Temperature:	40 °C					
<b>Injection Volume:</b>	0.5 µL					
Detection:	UV @ 254 nm	UV @ 254 nm (22 °C)				
Sample:	16-component	proprieta	ry test mixture			

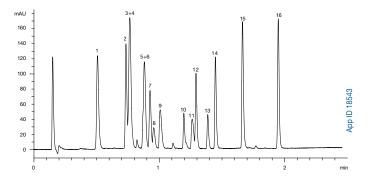
### Figure 3.

16-component test mixture using Kinetex<sup>™</sup> 2.6 μm C18 (50 x 2.1 mm) *without* KrudKatcher Ultra in-line filter



### Figure 4.

16-component test mixture using Kinetex 2.6 μm C18 (50 x 2.1 mm) *with* KrudKatcher Ultra in-line filter

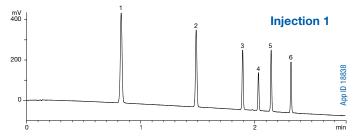


Finally, to illustrate the benefit of using the KrudKatcher Ultra in-line filter for extending column lifetime, a chromatographic separation of a ketone mixture on Kinetex 2.6  $\mu$ m PFP 50 x 2.1 mm column was set up on a UHPLC system (Figure 5). The initial injection (top) shows excellent peak shape and was maintained for over 1100 injections. However, after 1175 injections (middle) the chromatographic performance has noticeably degraded resulting in split peaks with some peak broadening and tailing evident. Simply replacing the KrudKatcher Ultra and re-injecting the sample (bottom) returns the original chromatographic performance. The KrudKatcher Ultra in-line filter successfully protected the ultra-high performance Kinetex column from damaging microparticulates in the mobile phase flow path and sample that would have dramatically shortened the column lifetime.

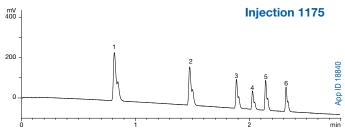
	Kinetex 2.6 µn 50 x 2.1 mm	n PFP			
Part No.:	00B-4477-AN				
	AF0-8497 (Kru	dKatcher Ul	tra In-Line	Filter)	
Mobile Phase:	A: Water				
	B: Methanol				
Gradient:	Time (min)	% B			
	0	30			
	2.9	100			
Flow Rate:	1.0 mL/min				
Temperature:	50 °C		Sample:	1. Acetophenone	4. Heptanophenone
Injection Volume:	0.2 µL			2. Butyrophenone	5. Octanophenone
Detection:	UV @ 254 nm	(22 °C)		3. Hexanophenone	6. Decanophenone

### Figure 5.

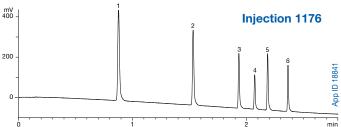
Initially, both the KrudKatcher Ultra in-line filter and Kinetex column show excellent performance



After extended use, the chromatography starts to degrade, producing split peaks



Replacing the KrudKatcher Ultra in-line filter extends the lifetime of the column



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### Conclusion

The use of KrudKatcher<sup>™</sup> Ultra in-line filters with ultra-high performance Kinetex<sup>™</sup> core-shell LC columns does not have a detrimental effect on chromatographic performance (retention time, peak width, resolution, or efficiency). However, the regular use of KrudKatcher Ultra protects and extends the lifetime of Kinetex columns by protecting them from damaging microparticulates that may be present in the sample and mobile phase. In addition, KrudKatcher Ultra offers similar protection for any ultrahigh performance column without increasing backpressure, or contributing excess void volume

### **References**

- 1. L. R. Snyder, and J.J. Kirkland; Introduction to Modern Liquid Chromatography, 2nd ed., John Wiley and Sons, Inc., New York, 1979, 228-230
- 2. U.D. Neue; HPLC Columns: Theory, Technology, and Practice, Wiley-VCH, New York, 1997, 349-350

### KrudKatcher Ultra In Line Filter

The KrudKatcher Ultra filter body houses an integrated 0.5 µm 316 stainless steel filter element that efficiently removes microparticulates from the flow stream without contributing to system backpressure or dead volume (<0.2 µL).



### **Ordering Information**

KrudKatcher Ultra In-Line Filter				
Part No.	Description	Unit		
AF0-8497	HPLC KrudKatcher Ultra Column In-Line Filter,	3/pk		
	0.5 µm Porosity x 0.004 in. ID			
Wrenches not provide	d KrudKatcher Illtra requires 5/e in wrench			

### **Ordering Information**

Kinetex™ 1.7 µm Minibore Columns (mm)					
50 x 2.1	100 x 2.1	150 x 2.1			
00B-4475-AN	00D-4475-AN	00F-4475-AN			
00B-4476-AN	00D-4476-AN	00F-4476-AN			
Minibore Columns (mm)					
50 x 2.1	100 x 2.1	150 x 2.1			
00B-4462-AN	00D-4462-AN	00F-4462-AN			
00B-4477-AN	00D-4477-AN	00F-4477-AN			
Kinetex™ 2.6 µm Solvent Saver MidBore™ Columns (mm)					
50 x 3.0	100 x 3.0	150 x 3.0			
00B-4462-Y0	00D-4462-Y0	00F-4462-Y0			
00B-4477-Y0	00D-4477-Y0	00F-4477-Y0			
Analytical Columns (mm	)				
50 x 4.6	100 x 4.6	150 x 4.6			
00B-4462-E0	00D-4462-E0	00F-4462-E0			
00B-4477-E0	00D-4477-E0	00F-4477-E0			
HILIC phase available, contact your Phenomenex technical consultant.					
		13			
	50 x 2.1 00B-4475-AN 00B-4476-AN Minibore Columns (mm) 50 x 2.1 00B-4462-AN 00B-4462-AN 00B-4477-AN Solvent Saver MidBore™ 50 x 3.0 00B-4462-Y0 00B-4477-Y0 Analytical Columns (mm 50 x 4.6 00B-4462-E0 00B-4477-E0	50 x 2.1         100 x 2.1           00B-4475-AN         00D-4475-AN           00B-4476-AN         00D-4476-AN           Minibore Columns (mm)         50 x 2.1           50 x 2.1         100 x 2.1           00B-4462-AN         00D-4462-AN           00B-4462-AN         00D-4462-AN           00B-4477-AN         00D-4477-AN           Solvent Saver MidBore™ Columns (mm)         50 x 3.0           00B-4462-YO         00D-4462-YO           00B-4477-YO         00D-4462-YO           00B-4477-YO         00D-4462-YO           00B-4462-FO         00D-4462-YO           00B-4462-FO         00D-4462-FO           00B-4462-FO         00D-4462-FO           00B-4462-FO         00D-4462-FO           00B-4477-FO         00D-4462-FO			



### UHPLC / HPLC Sure-Lok<sup>™</sup> High Pressure PEEK<sup>®</sup> **Male Nut Fittings**

Made of a proprietary PEEK blend, these ultra-high performance polymeric fittings are perfect for all but the most extreme highpressure applications, and best for ion- and bio-chromatography. High pressure nuts have a knurled surface designed to provide sufficient sealing force on the ferrule without wrenches. For 1/16 in. diameter tubing, there are two design types. The convenient onepiece design is pressure rated to 12,000 psi (827 bar) and stable up to temperatures of 200 °C. The second type is engineered as a 3-piece unit, with a ferrule and stainless steel gripping ring, that will provide leak-free connections up to 19,000 psi (1,310 bar). For higher pressure-rated fittings use the stainless steel nut and ferrule set (AQ0-8506).

AQ0-8503 Pressure rated up to 12,000 psi (827 bar)

AQ0-8504 shown with AQ0-8505 Pressure rated to 19,000 psi (1,310 bar)





### **Ordering Information**

Sure-Lok High Pressure PEEK Nuts				
Part No.	Description	Unit		
AQ0-8502	Sure-Lok High Pressure PEEK 1-Pc Nut, 10-32, for $^{1/_{16}}$ in. Tubing, 12,000 psi (827 bar)	2/pk		
AQ0-8503	Sure-Lok High Pressure PEEK 1-Pc Nut, 10-32, for $^{1/_{16}}$ in. Tubing, 12,000 psi (827 bar)**	10/pk		
AQ0-8504	Sure-Lok High Pressure PEEK Nut, 10-32, for 1/16 in. Tubing, 19,000 psi (1,310 bar) *.**	10/pk		
AQ0-8505	Sure-Lok PEEK Ferrule Assembly (2-pc), for High Pressure 2-Pc Nut (AQ0-8504)	10/pk		

\* Ferrule assembly (AQ0-8505) must be ordered separately.
\*\* Sure-Lok fitting tightening tool is required for AQ0-8503 and AQ0-8504

### Sure-Lok<sup>™</sup> Fitting Tightening Tool

Use this handy tool to tighten any standard, short- or long-style knurl-headed (high pressure) male nut like the ones above. The tool can also be used with many of the low-pressure nuts commonly used in the lab.



Sure-Lok Filling Tightening 1001			
Part No.	Description	Unit	
AQ0-8530	Sure-Lok Fitting Tightening Tool, Aluminum	ea	



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