A Comparison of Various Kinetex® C18 Phases

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Introduction

When performing reversed phase LC separations, there is a natural tendency of method developers to first test a C18 phase, the most commonly used phase in LC applications. Today, there are many different C18 phases available to method developers so choosing a simple C18 column may prove to be more challenging than one had originally thought.

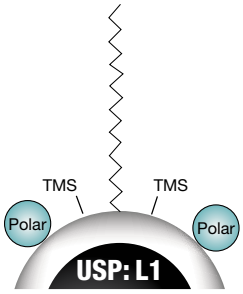
This technical note explores the different C18 phases within the Kinetex core-shell family of HPLC/UHPLC columns to determine the differences as well as to provide a general guideline of how to select the most appropriate C18 phase for a variety of applications.

Materials and Methods

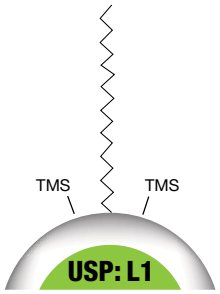
To provide a streamlined method development approach, the following cases were studied to determine the most appropriate C18 phase for each application:

- Acid, Base, Neutral Panel
- Highly Polar Analytes
- Highly Polar Bases (Catecholamines)
- Highly Polar Bases (Nucleotides)
- Hydrophobic Neutrals and Acids (Cannabinoids)

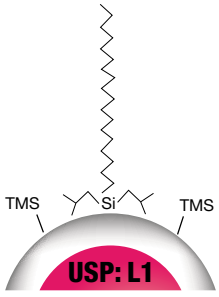
Three different Kinetex core-shell C18 phases were screened under the same conditions as well as optimized conditions in some cases:



Kinetex Polar C18
Combined C18 and polar modified surface that provide polar and non-polar retention alongside 100 % aqueous stability



Kinetex C18
Balanced C18 phase that provides the highest degree of hydrophobic selectivity relative to the other Kinetex phases



Kinetex XB-C18
Unique C18 phase that yields increased hydrogen bonding with hydrophobic selectivity, resulting in improved peak shape for basic compounds and increased retention of acidic compounds

Results and Discussion

I. Acid, Base, Neutral Panel using a Formic Acid Gradient

- In general, gradients will reduce selectivity differences between phases as the gradient rate itself will play a large role in analyte elution
- But, particularly when using formic acid and other weak buffers, differences in ion-exchange and polar interactions will still be apparent, giving subtle (or not so subtle) differences in selectivity
- These differences will be less apparent when using more strong buffer salts like phosphate, or when using ion-pairing agents (e.g. hexane sulfonic acid) or ion-masking agents (TEA; TFA)

LC Column: As specified
Dimensions: 50 x 4.6 mm
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile

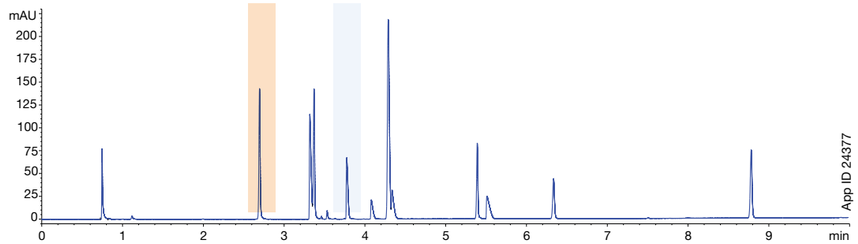
Gradient:

Time (min)	B (%)
0	5
0.5	5
10	95
11	95

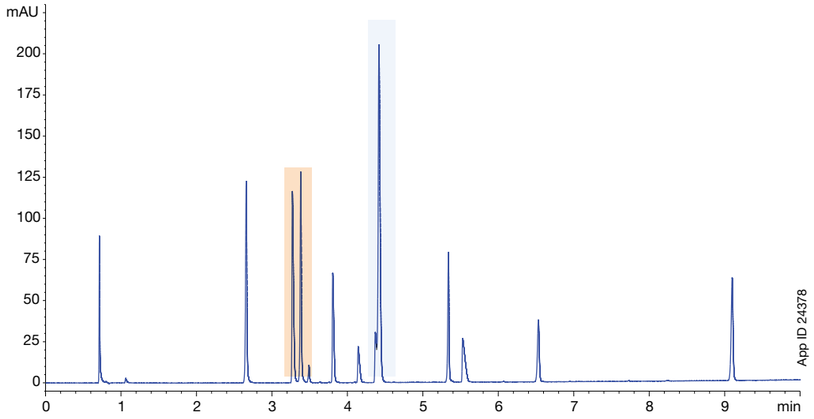
Flow Rate: 1.85 mL/min
Injection: 1 µL
Temperature: 30 °C

Sample:
1. Pyridine
2. Acetaminophen
3. Sulfathiazole
4. Quinidine
5. Quinidine Impurity
6. Acebutolol
7. Chlorpheniramine
8. Phenol
9. Triprolidine
10. Prednisolone
11. Nortriptyline
12. 5-Methylsalicaldehyde
13. Hexanophenone

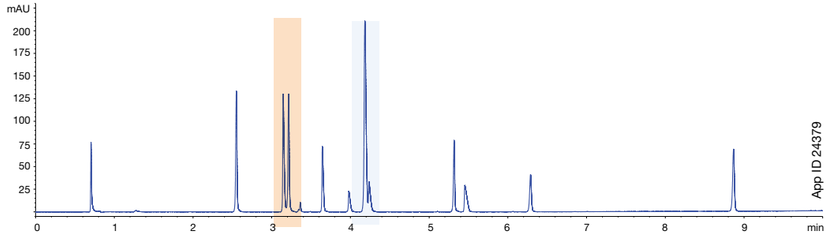
Kinetex® 2.6 µm Polar C18



Kinetex 2.6 µm C18



Kinetex 2.6 µm XB-C18

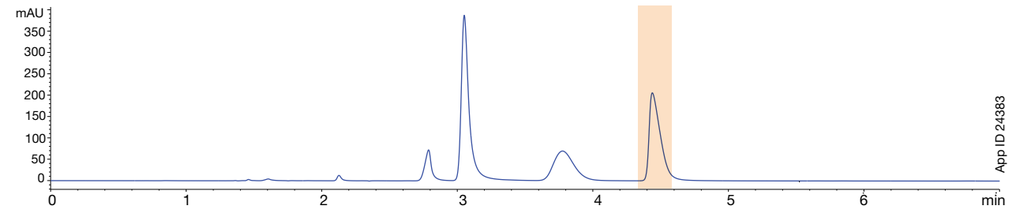


II. Highly Polar Analytes – Water-Soluble Vitamins

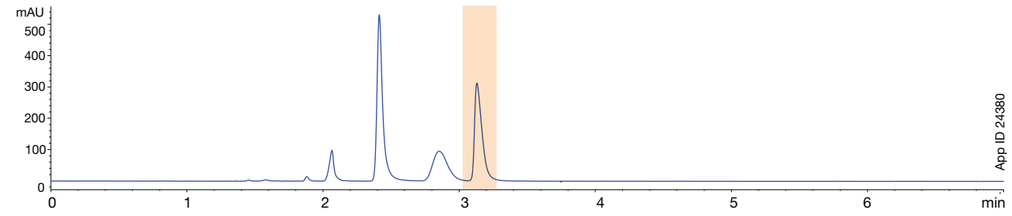
- Take advantage of the aqueous stability of Kinetex® Polar C18 to perform your analyses in 100 % aqueous conditions to maximize retention of polar analytes
- Traditional C18 phases like Kinetex C18 or XB-C18 should not be used below 2-3 % organic to maintain phase stability

LC Column: As specified
Dimensions: 50 x 4.6 mm
Mobile Phase: 20 mM Potassium phosphate, pH 3.5 / Methanol (97:3) (except where noted)
Flow Rate: 1 mL/min
Injection: 1 µL
Temperature: 35 °C
Detection: UV @ 210 nm
Sample:
1. Thiamine
2. Nicotinamide
3. Pyridoxal
4. Pyridoxine

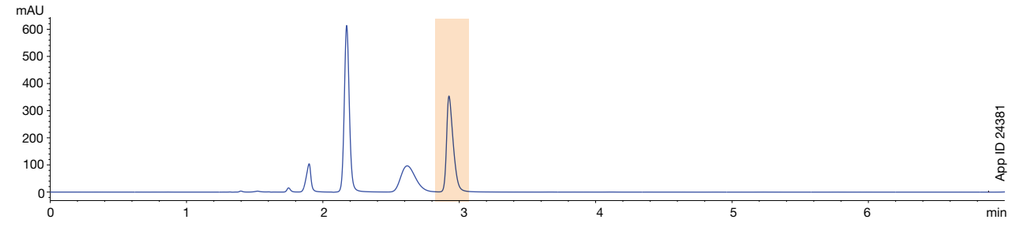
Kinetex 2.6 µm Polar C18 (mobile phase = 100% 20 mM Potassium phosphate, pH 3.5)



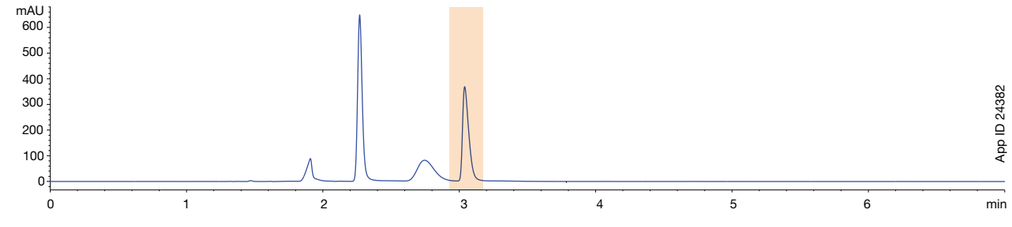
Kinetex 2.6 µm Polar C18



Kinetex 2.6 µm C18



Kinetex 2.6 µm XB-C18



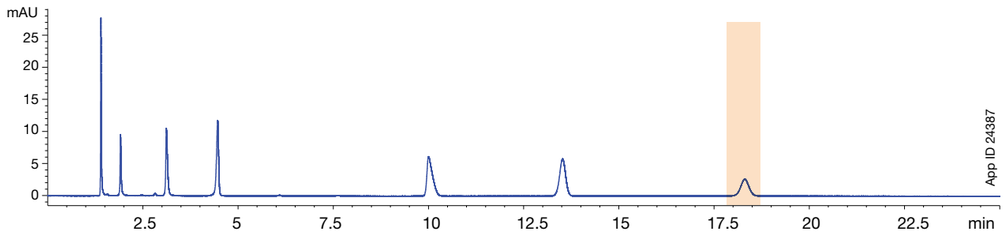
III. Highly Polar Bases – Catecholamines

- Take advantage of the aqueous stability on Kinetex® Polar C18 to perform your analyses in 100 % aqueous conditions to maximize retention of polar analytes
- Traditional C18 phases like Kinetex C18 or XB-C18 should not be used below 2-3 % organic to maintain phase stability

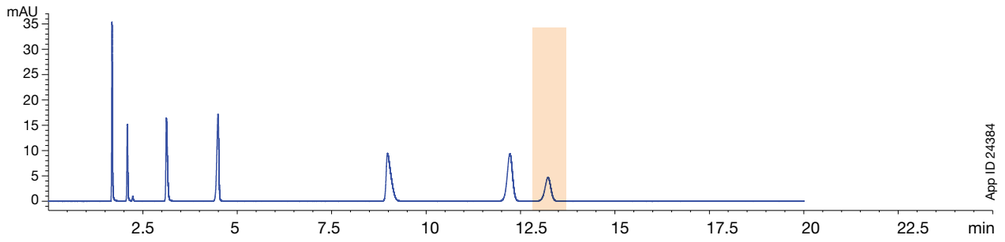
LC Column: As specified
Dimensions: 50 x 4.6 mm
Mobile Phase: 20 mM Ammonium acetate, pH 6.9 / Methanol (98:2) (except where noted)
Flow Rate: 1 mL/min
Injection: 1 µL
Temperature: Ambient
Detection: UV @ 280 nm

Sample:
1. Norepinephrine
2. Epinephrine
3. Dopamine
4. Serotonin
5. 5-Hydroxy-3-Indoleacetic Acid
6. 4-Hydroxy-3-Methoxyphenylacetic Acid

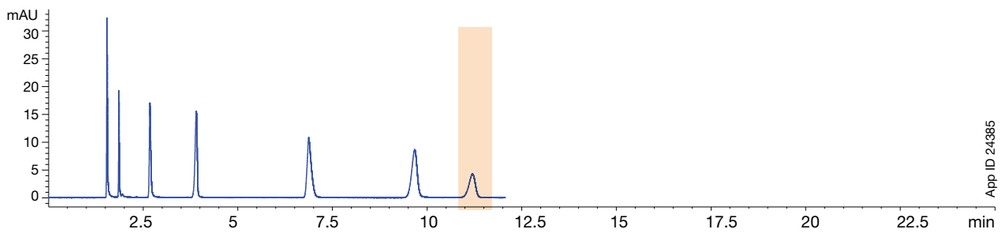
Kinetex 2.6 µm Polar C18 (mobile phase = 100 % 20 mM Ammonium acetate, pH 6.9)



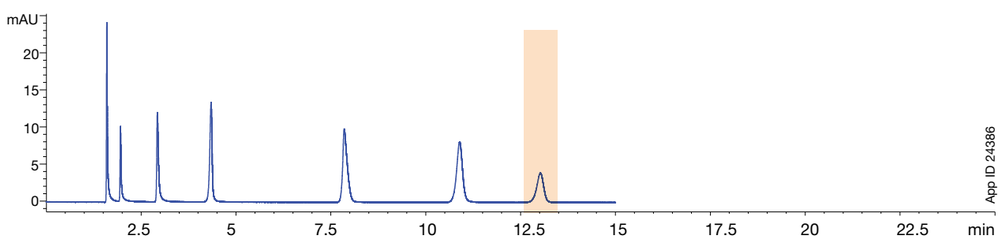
Kinetex 2.6 µm Polar C18



Kinetex 2.6 µm C18



Kinetex 2.6 µm XB-C18



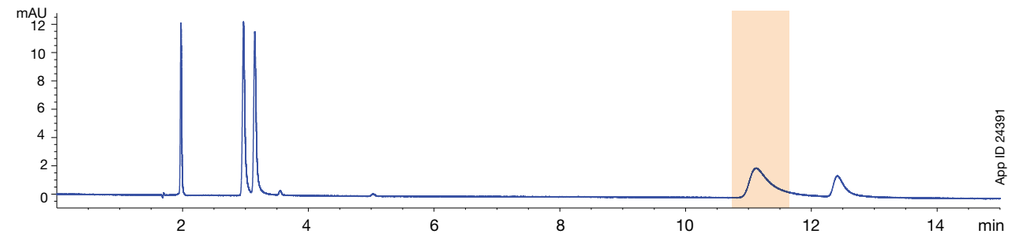
IV. Highly Polar Bases – Nucleotides

- Take advantage of the aqueous stability on Kinetex® Polar C18 to perform your analyses in 100 % aqueous conditions to maximize retention of polar analytes. The trade-off can be an increase in tailing, as is apparent below, but we include the data as a logical extreme; one would expect to optimize their method to find the right balance of retention and peak shape
- The key is that operating in highly aqueous conditions is only possible with the Kinetex Polar C18 phase

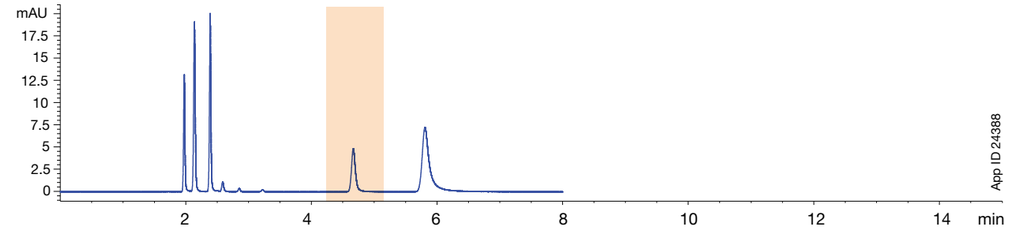
LC Column: As specified
Dimensions: 50 x 4.6 mm
Mobile Phase: 20 mM Ammonium acetate, pH 6.9 / Methanol (97:3) (except where noted)
Flow Rate: 1 mL/min
Injection: 1 µL
Temperature: Ambient
Detection: UV @ 280 nm

Sample:
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine
5. Guanosine-5-Monophosphate

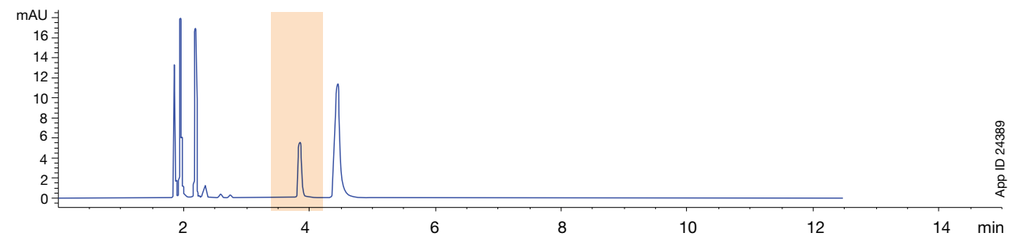
Kinetex 2.6 µm Polar C18 (mobile phase = 100 % 20 mM Ammonium acetate, pH 6.9)



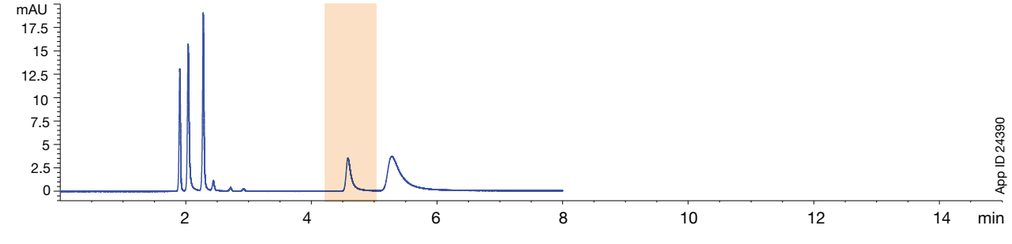
Kinetex 2.6 µm Polar C18



Kinetex 2.6 µm C18



Kinetex 2.6 µm XB-C18



V. Hydrophobic Neutrals and Acids – Cannabinoids

- Even very hydrophobic molecules will display selectivity differences on different C18 phases due to structural (e.g. positional isomers) or polar functional groups
- In this case, although selectivity was similar overall, the traditional Kinetex® C18 provided better selectivity for one critical pair (CBN and CBGA), which is interesting because they are totally different structurally one would think could easily separate

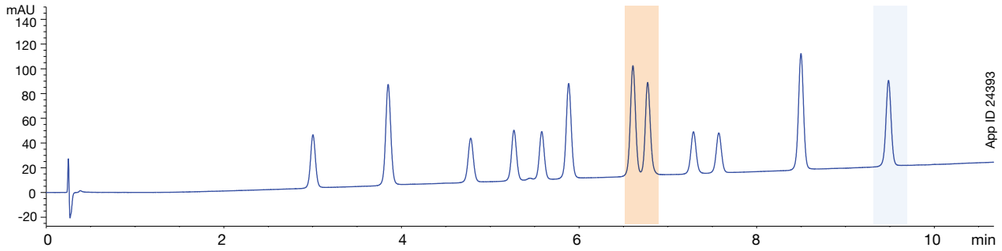
LC Column: As specified
Dimensions: 50 x 4.6 mm
Mobile Phase: A: 0.1 % Formic acid in Water
B:0.1 % Formic acid in Acetonitrile
Gradient:

Time (min)	B (%)
0	60
16	100

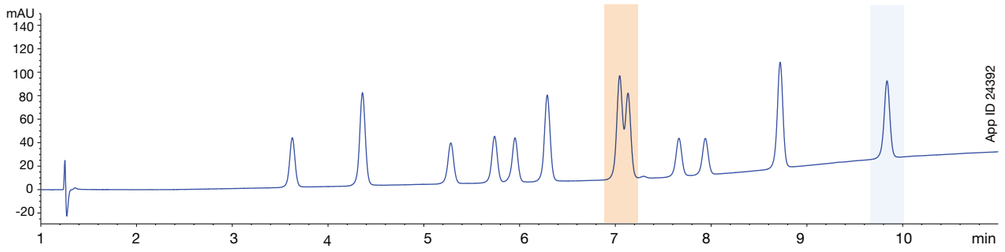
Flow Rate: 0.5 mL/min
Injection: 1 µL
Temperature: 50 °C
Detection: UV @ 230 nm

Sample:
1. CBDV
2. CBDVA
3. THC-V
4. CBD
5. CBG
6. CBDA
7. CBN
8. CBGA
9. D9-THC
10. D8-THC
11. CBC
12. THCA-A

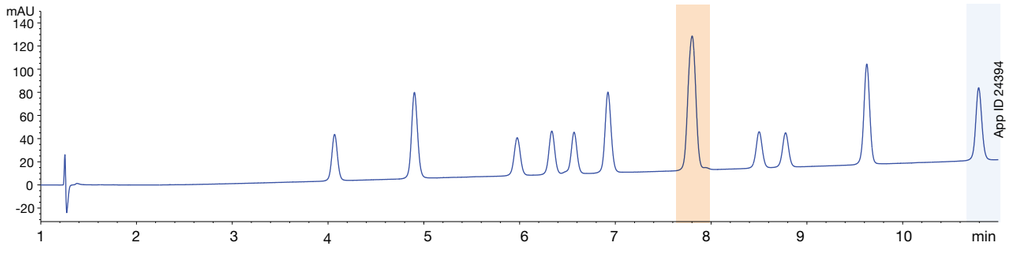
Kinetex 2.6 µm C18



Kinetex 2.6 µm Polar C18



Kinetex 2.6 µm XB-C18



Conclusions

- You will find differences in selectivity between the Kinetex® C18, Kinetex XB-C18, and Kinetex Polar C18 stationary phases based upon the differences in surface chemistry
- Under identical running conditions, the differences may be subtle – these are after all still all C18 phases – but subtle differences can make the difference between resolution and co-elution, or lead to reversals in elution order that can help to resolve a target analyte from matrix interferences
- With the newest Kinetex Polar C18 phase, however, you can operate in 100 % aqueous conditions, whereas the traditional C18 phases should not go below 2-3 % organic. This opens the potential to generate significant changes in retention for polar molecules.
- A thorough method development project should involve screening each of these phases, but if only one column could be screened, it is recommended that it be the Kinetex Polar C18, with its ability to operate in 100 % aqueous conditions, followed by either the Kinetex C18 or Kinetex XB-C18 to complement it
- The Kinetex Polar C18, with its aqueous stability and special surface modification, may provide a benefit for analyzing polar analytes, whereas the standard Kinetex C18 or XB-C18 phases, may be advantageous for separations based upon primarily hydrophobic differences
- Although it was not included in this study, our Kinetex EVO C18 is the obvious choice for operating in alkaline mobile phases, or when you need improved peak shape for tailing basic analytes, especially when using weakly-buffered mobile phases like 0.1 % formic acid

Kinetex Ordering Information

1.3 µm Minibore Columns (mm)		
Phases	**30 x 2.1**	**50 x 2.1**
C18	00A-4515-AN	00B-4515-AN

1.7 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	**30 x 3.0**	**50 x 3.0**	**100 x 3.0**	**3/pk**
XB-C18	00A-4498-Y0	00B-4498-Y0	00D-4498-Y0	AJ0-8775
C18	—	00B-4475-Y0	00D-4475-Y0	AJ0-8775
				for 3.0 mm ID

1.7 µm Microbore Columns (mm)			
Phases	**50 x 1.0**	**100 x 1.0**	**150 x 1.0**
EVO C18	00B-4726-A0	00D-4726-Y0	00F-4726-A0

1.7 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
EVO C18	—	00B-4726-AN	00D-4726-AN	00F-4726-AN	AJ0-9298
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	AJ0-8782
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782
					for 2.1 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**3/pk**
EVO C18	—	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	AJ0-9296
Polar C18	—	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	AJ0-9530
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
						for 4.6 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	**30 x 3.0**	**50 x 3.0**	**75 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
EVO C18	—	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
						for 3.0 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard™ ULTRA Cartridges‡
Phases	**30 x 2.1**	**50 x 2.1**	**75 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
EVO C18	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298
Polar C18	00A-4759-AN	00B-4759-AN	—	00D-4759-AN	00F-4759-AN	AJ0-9532
XB-C18	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
						for 2.1 mm ID

More Ordering Information →

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

APPLICATIONS

Kinetex® Ordering Information

2.6 Microbore Columns (mm)			
Phases	**50 x 1.0**	**100 x 1.0**	**150 x 1.0**
XB-C18	00B-4496-A0	00F-4496-E0	00F-4496-A0

3.5 µm Analytical Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	**100 x 4.6**	**150 x 4.6**	**3/pk**
XB-C18	00B-4496-A0	00D-4796-E0	AJ0-8768
			for 4.6 mm ID

5 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
EVO C18	00A-4633-AN	00B-4633-AN	00D-4633-AN	00F-4633-AN	AJ0-9298
XB-C18	00A-4605-AN	00B-4605-AN	00D-4605-AN	—	AJ0-8782
C18	00A-4601-AN	00B-4601-AN	00D-4601-AN	00F-4601-AN	AJ0-8782
					for 2.1 mm ID

5 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
EVO C18	00B-4633-Y0	00D-4633-Y0	00F-4633-Y0	AJ0-9297
XB-C18	00B-4605-Y0	00D-4605-Y0	00F-4605-Y0	AJ0-8775
C18	00B-4601-Y0	00D-4601-Y0	00F-4601-Y0	AJ0-8775
				for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
					for 4.6 mm ID

5 µm Semi-Preparative Columns (mm)			SecurityGuard SemiPrep Cartridges***
Phases	**150 x 10**	**250 x 10**	**10 x 10**
EVO C18	00F-4633-N0	00G-4633-N0	AJ0-9306
C18	00F-4601-N0	00G-4601-N0	AJ0-9278
			for 10 mm ID

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard PREP Cartridges*
Phases	**50 x 21.2**	**100 x 21.2**	**150 x 21.2**	**250 x 21.2**	**15 x 21.2**
EVO C18	00B-4633-P0-AX	00D-4633-P0-AX	00F-4633-P0-AX	00G-4633-P0-AX	AJ0-9304
XB-C18	00B-4605-P0-AX	00D-4605-P0-AX	00F-4605-P0-AX	00G-4605-P0-AX	AJ0-9145
C18	00B-4601-P0-AX	00D-4601-P0-AX	00F-4601-P0-AX	00G-4601-P0-AX	AJ0-9145
					for 21.2 mm ID

5 µm Axia Packed Preparative Columns (mm)					SecurityGuard PREP Cartridges**
Phases	**50 x 30**	**100 x 30**	**150 x 30**	**250 x 30**	**15 x 30**
EVO C18	00B-4633-U0-AX	00D-4633-U0-AX	00F-4633-U0-AX	00G-4633-U0-AX	AJ0-9305
XB-C18	00B-4605-U0-AX	00D-4605-U0-AX	00F-4605-U0-AX	00G-4605-U0-AX	AJ0-9204
C18	00B-4601-U0-AX	00D-4601-U0-AX	00F-4601-U0-AX	00G-4601-U0-AX	AJ0-9204
					for 30 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277
*** SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-9281

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guarantee

If Kinetex core-shell analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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