

## Rapid Analysis of Hop Acids in Beer using Strata™-X Solid Phase Extraction and Kinetex® 2.6 µm Core-Shell Technology Column

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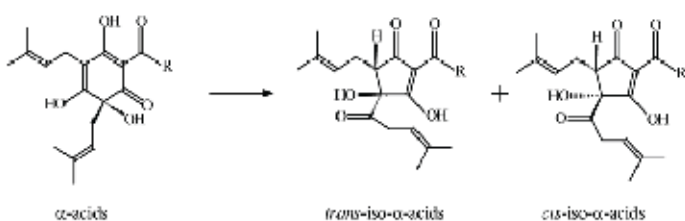
An improved HPLC analysis for iso-alpha hop acids in beer is demonstrated using the Kinetex 2.6 µm core-shell column, resulting in improved peak shape, easier quantitation, and reduced analysis times. Sample cleanup using Strata-X can also remove many potential interferences in the beer matrix, resulting in cleaner samples for HPLC analysis.

### Introduction

Iso-alpha acids, derived from hops, are the compounds responsible for beer's bitter taste. During the beer brewing process compounds known as alpha acids are extracted from the hops and isomerized to form iso-alpha acids (**Figure 1**). The conversion of alpha acids into iso-alpha acids takes place when the hops are added to the wort (unfinished beer) and boiled. The amount and type of iso-alpha acids formed is dependent on a number of factors including the boiling time, the variety and age of the hops, and the pH of the wort. The bitterness derived from iso-alpha hop acids is a primary flavor attribute of beer and accurate determination of beer bitterness is of great importance to the brewer. Therefore, to maintain a consistent product, brewers must carefully monitor the levels of iso-alpha acids throughout the manufacturing process and in the final beer product.

Iso-alpha acid levels are typically monitored using reversed phase HPLC. Until recently, these analyses have been performed using columns packed with fully porous silica particles, with run times of 15-20 minutes or longer. By switching to HPLC columns packed with Kinetex core-shell particles, these analyses can be significantly improved and performed in a fraction of the currently accepted analysis time.

**Figure 1.**  
Structures of Iso-Alpha Acids



R =	α-acids	trans-iso-α-acids	cis-iso-α-acids
CH(CH <sub>3</sub> ) <sub>2</sub>	cohumulone	trans-isocohumulone	cis-isocohumulone
CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	humulone	trans-isohumulone	cis-isohumulone
CH(CH <sub>3</sub> )C <sub>2</sub> H <sub>5</sub>	adhumulone	trans-isoadhumulone	cis-isoadhumulone

### Materials and Methods

#### Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. HPLC Grade methanol and acetonitrile were purchased from Honeywell, Burdick & Jackson (Muskegon, MI), Milli-Q® water was used for solid-phase extraction and sample-preparation. HPLC Grade water was purchased from Honeywell, Burdick & Jackson and used to prepare the LC mobile phase.

#### Standards

The hop standards were purchased from the American Society of Brewing Chemists. The standards came in two mixtures: one contained the normal iso-alpha acids (Isocohumulone, Isohumulone, and Isoadhumulone) and the other contained the reduced tetrahydroiso-alpha acids (Tetrahydroisocohumulone, Tetrahydroisohumulone, and Tetrahydroisoadhumulone). The tetrahydroiso-alpha acid standard mix contains both the cis and trans isomers of each compound.

#### Equipment and Materials

Agilent® 1100 Series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with quaternary pump, autosampler, column oven, and variable wavelength detector.

#### Solid Phase Extraction

Each beer sample was degassed by stirring for approximately 30 min at room temperature.

<b>Cartridge:</b>	Strata-X 200 mg/6 mL
<b>Part No.:</b>	8B-S100-FCH
<b>Condition:</b>	4 mL acidified methanol (1-2 mL/min)
<b>Equilibrate:</b>	4 mL water (1-2 mL/min) Note: Do not let sorbent run dry
<b>Load:</b>	5 mL of beer degassed (1 mL/min)
<b>Wash:</b>	4 mL of 40 % methanol in water
<b>Dry:</b>	>10" Hg for 5 minutes to remove residual water
<b>Elute:</b>	2 mL of acidified methanol (1 mL/min)
<b>Drydown:</b>	Nitrogen gas at 55 °C
<b>Reconstitute:</b>	500 µL of mobile phase

### HPLC Conditions

<b>Column:</b>	Kinetex® 2.6 µm C18 100 Å
<b>Dimensions:</b>	as noted
<b>Mobile Phase:</b>	Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
<b>Flow Rate:</b>	1.4 mL/min
<b>Injection Volume:</b>	as noted
<b>Temperature:</b>	as noted
<b>Detection:</b>	UV @ 270 nm (ambient)
<b>Sample:</b>	as noted

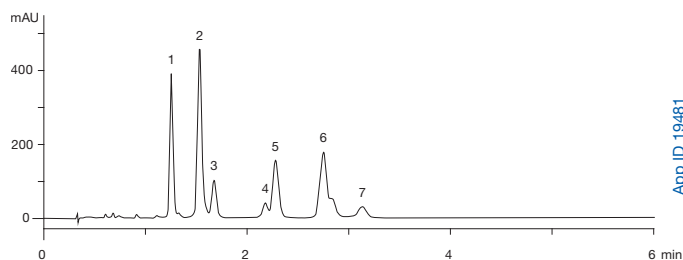
### Results and Discussion

**Figure 2** is a chromatogram of hop acid standards (obtained from the American Society of Brewing Chemists) run on a Kinetex 2.6 µm C18 column. All six of the iso-alpha acids that are most commonly tested for by brewers, were resolved isocratically in less than 7 min. The first three hop acids isomers (isocohumulone, isohumulone, and isoadhumulone) are derived from the naturally occurring alpha-acids in hops. The last three hop acids isomers (tetrahydroisocohumulone, tetrahydroisohumulone, and tetrahydroisoadhumulone) are specially modified reduced forms of the corresponding iso-alpha acids (tetrahydro-iso-alpha acids) and resistant to photo degradation. Both the naturally derived and the tetrahydro-iso-alpha acids have cis and trans isomers, therefore partial or even full resolution of some of the cis and trans iso-alpha acids is to be expected.

Both the 50 mm and 100 mm Kinetex columns completely separate all 6 iso-alpha acids (**Figures 2a** and **2b**). The 100 mm column provides near baseline separation for cis- and trans-tetrahydroisocohumulone (peaks 4 & 5), while the 50 mm column only partially separates these compounds. Cis- and trans-tetrahydroisohumulone coelute on both Kinetex column dimensions (peak 6), but because of the better separation of the tetrahydroisocohumulone isomers, more accurate quantitation is achieved with the longer 100 mm Kinetex column. It is easy to see that the separation with the Kinetex column is substantially improved versus the fully porous C18 column; providing faster separation and improved resolution and peak shape for all compounds. The chromatographic resolution for all of the hop acids, in particular for isohumulone and isoadhumulone, is much better on the Kinetex column. This increases the ability to more accurately monitor the brewing process and the final beer product.

**Figure 2.**  
Iso-Alpha Acid Standards

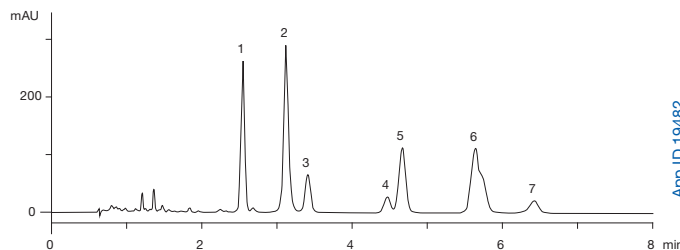
a) Kinetex 2.6 µm C18 50 x 4.6 mm



App ID 19481

**Column:** Kinetex 2.6 µm C18 100 Å  
**Dimensions:** 50 x 4.6 mm  
**Part No.:** 00B-4462-E0  
**Mobile Phase:** Methanol / Water / Phosphoric acid (75:24:1, v/v/v)  
**Flow Rate:** 1.4 mL/min  
**Injection Volume:** 1 µL  
**Temperature:** 45 °C  
**Detection:** UV @ 270 nm (ambient)  
**Sample:** 1. Isocohumulone  
 2. Isohumulone  
 3. Isoadhumulone  
 4. *trans*-Tetrahydroisocohumulone  
 5. *cis*-Tetrahydroisocohumulone  
 6. Tetrahydroisohumulone  
 7. Tetrahydroisoadhumulone

b) Kinetex 2.6 µm C18 100 x 4.6 mm



App ID 19482

**Column:** Kinetex 2.6 µm C18 100 Å  
**Dimensions:** 100 x 4.6 mm  
**Part No.:** 00D-4462-E0  
**Mobile Phase:** Methanol / Water / Phosphoric acid (75:24:1, v/v/v)  
**Flow Rate:** 1.4 mL/min  
**Injection Volume:** 1 µL  
**Temperature:** 45 °C  
**Detection:** UV @ 270 nm (ambient)  
**Sample:** 1. Isocohumulone  
 2. Isohumulone  
 3. Isoadhumulone  
 4. *trans*-Tetrahydroisocohumulone  
 5. *cis*-Tetrahydroisocohumulone  
 6. Tetrahydroisohumulone  
 7. Tetrahydroisoadhumulone

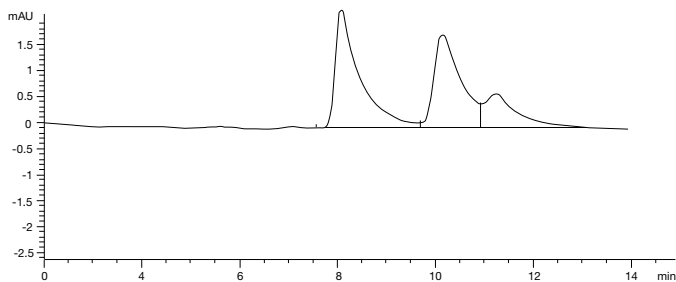
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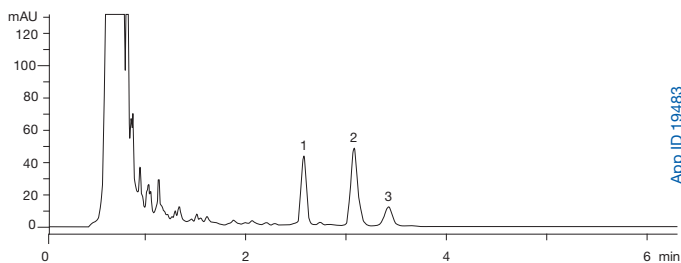
**Figure 3a** is a customer supplied chromatogram of an analysis of a beer product using a conventional, fully porous packing. As evidenced in the chromatogram, the peak shapes for these acids are poor and isohumulone and isoalhumulone are not well resolved. When a similar analysis is performed on a beer sample (Red Stripe® Jamaican Lager) containing the same iso alpha-acids using Kinetex 2.6 µm C18 (**Figure 3b**), all of the hop acids are well resolved with excellent peak shape. In addition, the run time has been reduced from ~14 to less than 4 minutes.

**Figure 3.**  
Analysis of Iso-Alpha Acids from commercial beer sample

a) Fully porous 5 µm C18 150 x 4.6 mm



b) Kinetex® 2.6 µm C18 100 x 4.6 mm



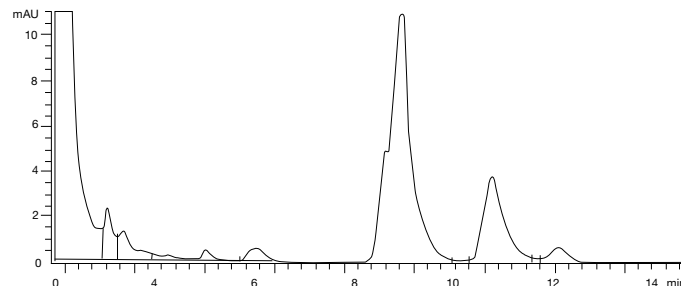
App ID 19483

**Column:** Kinetex 2.6 µm C18 100 Å  
**Dimensions:** 100 x 4.6 mm  
**Part No.:** 00D-4462-E0  
**Mobile Phase:** Methanol / Water / Phosphoric acid (75:24:1, v/v/v)  
**Flow Rate:** 1.4 mL/min  
**Injection Volume:** 50 µL  
**Temperature:** 22 °C  
**Detection:** UV @ 270 nm (ambient)  
**Sample:** 1. Isohumulone  
 2. Isohumulone  
 3. Isoalhumulone

**Figures 4a** and **4b** are chromatograms obtained from the analysis of beer samples that contain tetrahydro-iso-alpha acids. The customer supplied chromatogram (**Figure 4a**) obtained using an HPLC column packed with fully porous particles shows that the tetrahydro-iso-alpha hop acids exhibit significant peak tailing and poor resolution, and the total analysis time is about 12 minutes. However, when a similar sample (Miller Genuine Draft; **Figure 4b**) is analyzed using the Kinetex 2.6 µm C18 column, the tetra acids are eluted in less than 7 minutes. Note that the cis and trans isomers are partially resolved under these conditions, but quantified as a single unit.

**Figure 4.**  
Analysis of Miller® Genuine Draft beer samples containing Tetrahydro-iso-alpha Acids

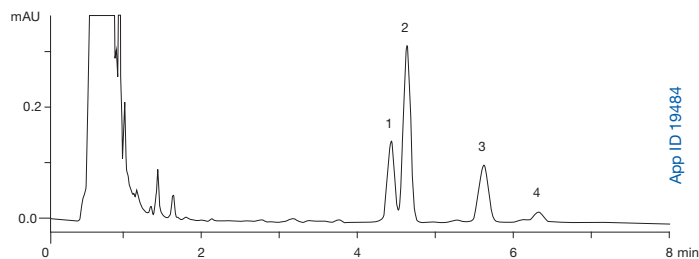
a) Fully porous 5 µm C18 150 x 4.6 mm



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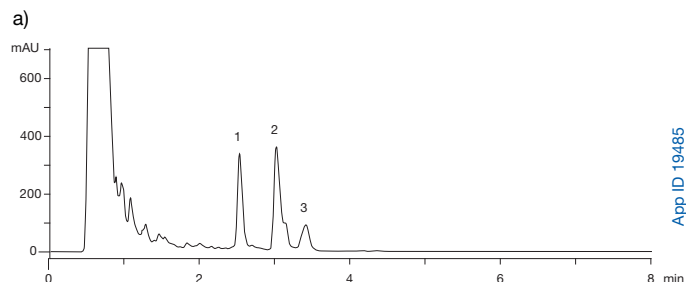
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b) Kinetex 2.6 µm C18 100 x 4.6 mm



**Column:** Kinetex 2.6 µm C18 100 Å  
**Dimensions:** 100 x 4.6 mm  
**Part No.:** 00D-4462-E0  
**Mobile Phase:** Methanol / Water / Phosphoric acid (75:24:1, v/v/v)  
**Flow Rate:** 1.4 mL/min  
**Injection Volume:** 50 µL  
**Temperature:** 22 °C  
**Detection:** UV @ 270 nm (ambient)  
**Sample:** 1. *trans*-Tetrahydroisochumulone  
 2. *cis*-Tetrahydroisochumulone  
 3. Tetrahydroisohumulone  
 4. Tetrahydroisoadhumulone

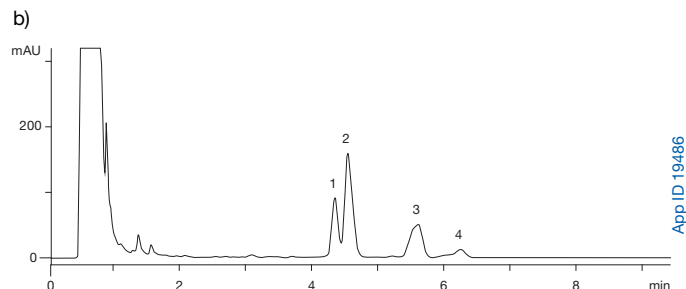
**Figure 5.**  
 Iso-Alpha Acids from Red Stripe® beer following SPE



**Column:** Kinetex 2.6 µm C18 100 Å  
**Dimensions:** 100 x 4.6 mm  
**Part No.:** 00D-4462-E0  
**Mobile Phase:** Methanol / Water / Phosphoric acid (75:24:1, v/v/v)  
**Flow Rate:** 1.4 mL/min  
**Injection Volume:** 50 µL  
**Temperature:** 22 °C  
**Detection:** UV @ 270 nm (ambient)  
**Sample:** 1. Isochumulone  
 2. Isohumulone  
 3. Isoadhumulone

The iso-alpha acids are present in sufficient concentration to be able to be detected by simply injecting 50 µL of degassed beer straight onto the LC column without any sample preparation (other than degassing). All analytes are completely separated from any obvious matrix interferences. However, the complex nature of the beer matrix makes sample preparation worth investigating. The iso-alpha acids were successfully extracted from packaged beer using Strata™-X SPE products. A 40 % methanol wash was found to remove some sample matrix components without adversely affecting analyte recovery. Two mL of acidified methanol was sufficient for eluting the analytes from the SPE cartridge. Recoveries from two commercial beer samples are shown in **Tables 1** and **2** and the corresponding chromatograms are shown in **Figure 5**.

Tetrahydro-iso-alpha acids from Miller® Genuine Draft beer following SPE



**Column:** Kinetex 2.6 µm C18 100 Å  
**Dimensions:** 100 x 4.6 mm  
**Part No.:** 00D-4462-E0  
**Mobile Phase:** Methanol / Water / Phosphoric acid (75:24:1, v/v/v)  
**Flow Rate:** 1.4 mL/min  
**Injection Volume:** 50 µL  
**Temperature:** 22 °C  
**Detection:** UV @ 270 nm (ambient)  
**Sample:** 1. *trans*-Tetrahydroisochumulone  
 2. *cis*-Tetrahydroisochumulone  
 3. Tetrahydroisohumulone  
 4. Tetrahydroisoadhumulone

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**Table 1.**

SPE Recoveries of Iso-alpha Acids from Red Stripe® Jamaican Lager Beer using Strata™-X

Iso-Alpha Acids	% Recovery
Isocohumulone	104
Isohumulone	104
Isoadhumulone	118

**Table 2.**

SPE Recoveries of Tetrahydro-Iso-alpha Acids from Miller® Genuine Draft Beer using Strata-X

Tetra Iso-alpha Acids	% Recovery
Tetrahydroisocohumulone	80
Tetrahydroisohumulone	82
Tetrahydroisoadhumulone	69

### Conclusions

Typical HPLC methods for measuring iso-alpha acids throughout the brewing process have relied upon HPLC columns packed with fully porous particles. Converting these methods over to columns packed with high efficiency Kinetex® 2.6 µm core-shell particles significantly improves chromatographic resolution while drastically reducing analysis times. While the iso-alpha acids are typically present in sufficient concentration to allow for direction analysis of the beer sample, a simple SPE cleanup was demonstrated to remove potential matrix interferences with good recovery.

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## APPLICATIONS

### Ordering Information

#### Sample Preparation Vacuum Manifolds

Part No.	Description	Unit
AHO-6024	SPE 24-Position Vacuum Manifold Set, complete assembly	ea
AHO-6023	SPE 12-Position Vacuum Manifold Set, complete assembly	ea
AHO-7502	SPE 10-Position Tall-Boy™ Vacuum Manifold Set, complete assembly	ea
AHO-7284	96-Well Plate Manifold, Acrylic	ea

#### Vacuum Manifold Accessories

Part No.	Description	Unit
AHO-7191	Adapter Caps for 1, 3 and 6 mL SPE tubes, polyethylene, with Luer tip	15/pk
AHO-7378	Adapter Caps for 12 and 20 mL SPE tubes, polyethylene, with Luer tip	5/pk
AHO-7379	Adapter Caps for 60 mL SPE tubes, polyethylene, with Luer tip	5/pk
AHO-8278	Strata Syringe and Adapter Kit	ea
AHO-6034	SPE Manifold Needles, polypropylene	24/pk
AHO-6035	SPE Manifold Needles, stainless steel	12/pk
AHO-6050	SPE Drying Attachment for 12-position manifold	ea
AHO-6051	SPE Drying Attachment for 24-position manifold	ea
AHO-6053	Female Luer Fittings	2/pk
AHO-6054	Male Luer Fittings	2/pk
AHO-6057	Vacuum Gauge and Valve Assembly	ea
AHO-6062	Control Valve, Teflon®	25/pk
AHO-6064	Teflon Needles	100/pk
AHO-6065	Teflon Needles	500/pk

#### Strata™-X

Sorbent Mass	Part No.	Unit
<b>Tube</b>		
30 mg	8B-S100-TAK	1 mL (100/box)
30 mg	8B-S100-TBJ	3 mL (50/box)
60 mg	8B-S100-UBJ	3 mL (50/box)
100 mg	8B-S100-EBJ	3 mL (50/box)
100 mg	8B-S100-ECH	6 mL (30/box)
200 mg	8B-S100-FBJ	3 mL (50/box)
200 mg	8B-S100-FCH	6 mL (30/box)
500 mg	8B-S100-HBJ	3 mL (50/box)
500 mg	8B-S100-HCH	6 mL (30/box)
<b>Giga™ Tube</b>		
500 mg	8B-S100-HDG	12 mL (20/box)
1 g	8B-S100-JDG	12 mL (20/box)
1 g	8B-S100-JEG	20 mL (20/box)
2 g	8B-S100-KEG	20 mL (20/box)
5 g	8B-S100-LFF	60 mL (16/box)
<b>96-Well Plate</b>		
10 mg	8E-S100-AGB	2 Plates/Box
30 mg	8E-S100-TGB	2 Plates/Box
60 mg	8E-S100-UGB	2 Plates/Box

Additional sizes available. Contact your Phenomenex Sample Preparation Specialist for additional information.

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### Kinetex® Ordering Information

#### 2.6 µm Analytical Columns (mm)

KrudKatcher™  
Ultra In-Line Filter\*

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	/3pk
<b>XB-C18</b>	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AF0-8497
<b>C18</b>	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AF0-8497
<b>C8</b>	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AF0-8497
<b>PFP</b>	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AF0-8497
<b>HILIC</b>	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AF0-8497

#### 2.6 µm MidBore™ Columns (mm)

KrudKatcher  
Ultra In-Line Filter\*

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	/3pk
<b>XB-C18</b>	—	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	—	AF0-8497
<b>C18</b>	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AF0-8497
<b>C8</b>	—	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	—	AF0-8497
<b>PFP</b>	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0	AF0-8497
<b>HILIC</b>	00A-4461-Y0	—	—	—	00F-4461-Y0	AF0-8497

#### 2.6 µm Minibore Columns (mm)

KrudKatcher  
Ultra In-Line Filter\*

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	/3pk
<b>XB-C18</b>	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AF0-8497
<b>C18</b>	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AF0-8497
<b>C8</b>	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN	AF0-8497
<b>PFP</b>	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AF0-8497
<b>HILIC</b>	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AF0-8497

#### 1.7 µm Minibore Columns (mm)

KrudKatcher  
Ultra In-Line Filter\*

	50 x 2.1	100 x 2.1	150 x 2.1	/3pk
<b>XB-C18</b>	00B-4498-AN	00D-4498-AN	—	AF0-8497
<b>C18</b>	00B-4475-AN	00D-4475-AN	00F-4475-AN	AF0-8497
<b>C8</b>	00B-4499-AN	00D-4499-AN	—	AF0-8497
<b>PFP</b>	00B-4476-AN	00D-4476-AN	00F-4476-AN	AF0-8497
<b>HILIC</b>	00B-4474-AN	—	—	AF0-8497

\*KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

### UHPLC / HPLC Sure-Lok™ High Pressure PEEK™ Male Nut Fittings

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

# TN-1085 APPLICATIONS

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Strata-X is patented by Phenomenex, Inc. U.S. Patent No. 7,119,145

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