APPLICATIONS

Comparison of Two Particle Morphologies and Four C18 Phases When Applied to the Underivatized Retention of Five Carboxylic Acids Under Typical Reversed Phase LC Conditions and UV-Vis Detection

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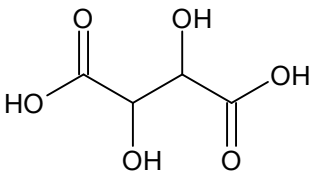
Introduction

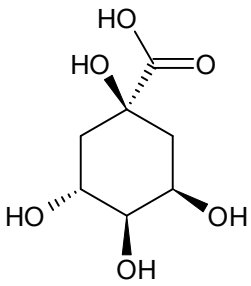
The prevalent use and industrial application of organic acids has driven the interest in developing a reversed phase HPLC method able to separate and analyze multiple acids, simultaneously, and in a short amount of time. The most common organic acids contain one or more carboxylic acid functionality and this determines the relative acidity or pK_a of the acid. The pK_a of the acidic hydrogen is a function of the adjacent groups to it and a result of the degree of intermolecular conjugation with electron-withdrawing groups also present within the compound. This dependency on conjugation results in a range of potential pK_a values covering this group of organic acids and translates into a range of polarities and shifts in lipophilicities across these molecules. When this is coupled with the fact that most organic acids are already polar, to some degree, under reversed phase conditions, adequate retention without derivatization or ion-pairing additives can present a significant challenge. Additionally, achieving baseline separation of closely related acids all the while ensuring good overall peak shape whilst striving for short analysis, can make time difficult.

This application investigates the relative selectivity of four different alkyl C18 stationary phases all containing different covalently bonded polar groups in addition to their C18 functionality. The initial screening conditions were the same for all columns and utilized a 100 % aqueous mobile phase without the use of any derivatization to the acids or the addition of ion-pairing agents. The goal of this application was to demonstrate a simple yet effective separation of five common organic acids under typical reversed phase conditions amenable to all laboratory situations. Polar stationary phase modifications, as well as embedded charged functionalities, were also included in this study. The effect of particle morphology on the separation of five organic acids were within the scope of this investigation (**Table 1**). For this study, we evaluated Kinetex® core-shell (superficially porous) and Luna® Omega fully porous particles and looked at the same stationary phase chemistries bonded to each. After selecting the most relevant stationary phase and particle size, flow rate and column length were adjusted to optimize chromatographic effect and performance.

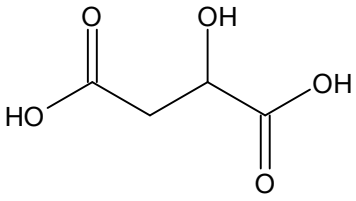
Tartaric Acid



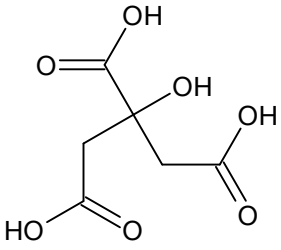
Quinic Acid



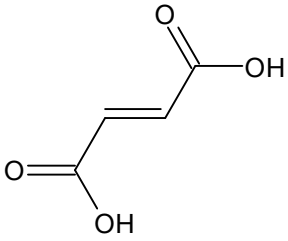
Malic Acid



Citric Acid



Fumaric Acid



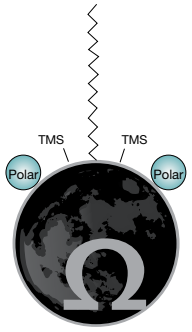
	Tartaric Acid	Quinic Acid	Malic Acid	Citric Acid	Fumaric Acid
Mol. Weight	150.09 g/mol	192.17 g/mol	134.09 g/mol	192.12 g/mol	116.07 g/mol
Acidic pK_a	2.72	3.46	3.20, 5.13	3.05	3.35, 4.22
LogP	-1.83	-2.70	1.11	-1.32	-0.04

Experimental

Analytical reference standards for Tartaric, Quinic, Malic, Citric, and Fumaric acid were obtained through Sigma-Aldrich® (St. Louis, MO). All phases were run under identical conditions on the same system and at the same time period. An Agilent® (Santa Clara, CA) 1100 Infinity HPLC system was used for this investigation with the UV-Vis detection set to 226 nm, and a reference wavelength of 360 nm. A standard solution was prepared in a diluent of water at a concentration of 1 mg/mL all except Fumaric acid (0.005 mg/mL) and an injection volume of 2 µL was used. The mobile phase used was 100 % Water with 100 mM Potassium Phosphate buffer pH adjusted to 2.5. For initial screening conditions, a flow rate of 1.5 mL/min was evaluated.

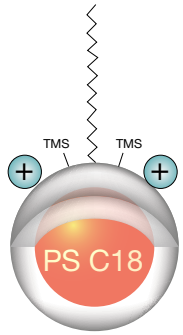
All columns used were from Phenomenex, Inc. (Torrance, CA) and the phases selected for this work were Luna Omega Polar C18, Luna Omega PS C18, Kinetex Polar C18, and Kinetex PS C18.

Table 1.
Material Characteristics



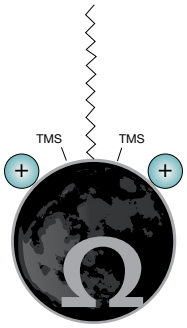
Luna® Omega Polar C18

100 % aqueous stability and enhanced selectivity/retention for polar analytes without diminishing useful non-polar retention. The C18 ligand provides general hydrophobic interactions while a polar modified particle surface provides enhanced polar compound retention.



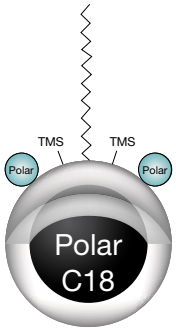
Kinetex® PS C18

A multi-interaction, 100 % aqueous stable C18 column with a positive surface modification that demonstrates unique selectivity and improved peak shape for basic compounds.



Luna Omega PS C18

Unique, 100 % aqueous stable mixed-mode phase that provides both polar and non-polar retention. The surface contains a positive charged ligand which aids in the retention of acidic compounds through ionic interactions, while the C18 ligand promotes general reversed phase hydrophobic retention. The positively charged surface also improves basic compound peak shape through ionic repulsion.



Kinetex Polar C18

Combined C18 and polar modified surface that provides polar and non-polar retention alongside 100 % aqueous stability.

Packing material	Available particle size (µm)	Pore size (Å)	Effective surface area (m^2/g)	Effective carbon load (%)	pH stability
Luna Omega Polar C18	1.6, 3, 5	100	260	9	1.5 – 8.5*
Luna Omega PS C18	1.6, 3, 5	100	260	9	1.5 – 8.5*
Kinetex PS C18	2.6	100	200	9	1.5 – 8.5*
Kinetex Polar C18	2.6	100	200	9	1.5 – 8.5*

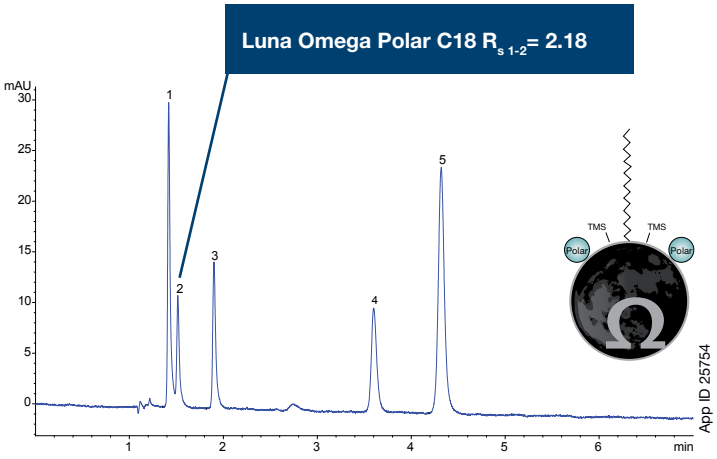
*pH stability under gradient conditions. pH stability is 1.5 – 10.0 under isocratic conditions.

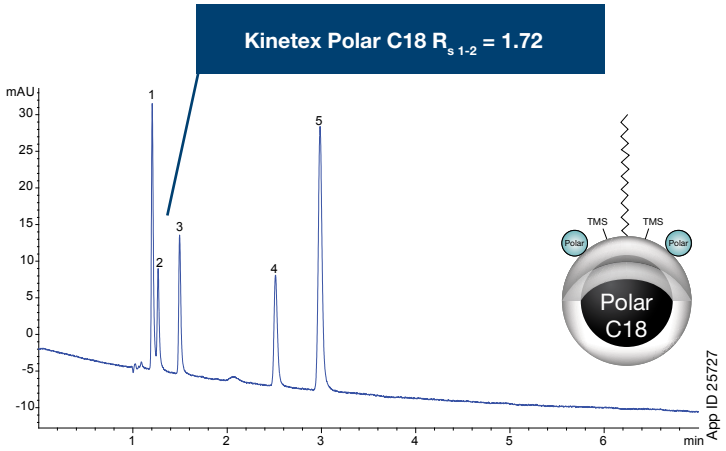
Figure 1.
Selectivity screening of polar organic acids on four alkyl columns of 150 x 4.6 mm dimension

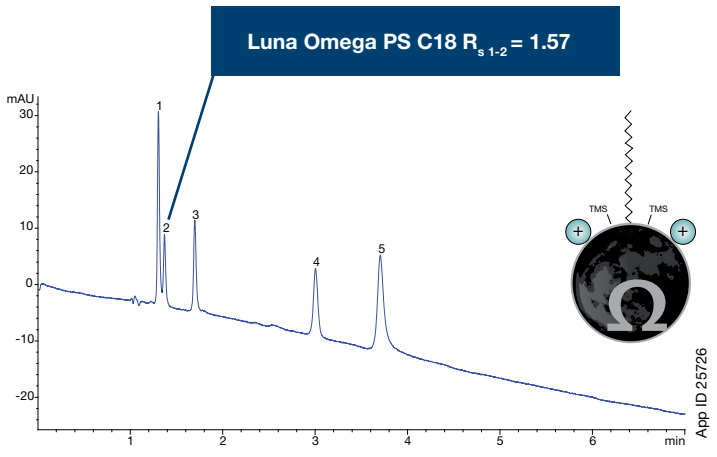
Conditions for all columns:

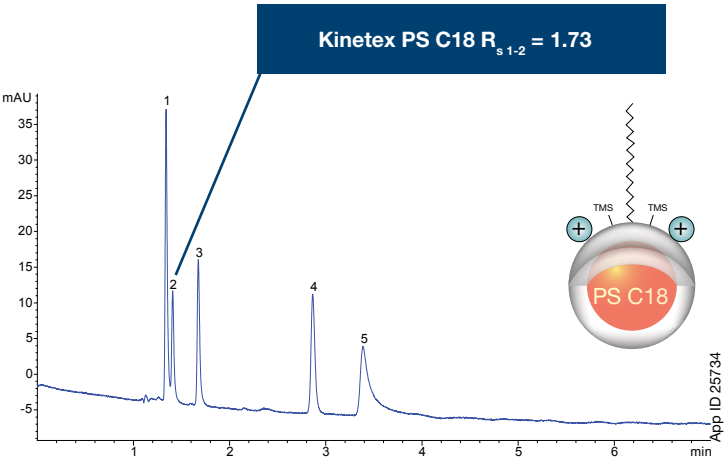
Column: Luna® Omega 3 µm Polar C18
Luna Omega 3 µm PS C18
Kinetex® 2.6 µm Polar C18
Kinetex 2.6 µm PS C18
Dimensions: 150 x 4.6 mm
Mobile Phase: Water with 100 mM Potassium Phosphate Monobasic (pH 2.5)

Flow Rate: 1.5 mL/min
Temperature: 35 °C
Detection: UV-Vis @ 226 nm
Sample:
1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid





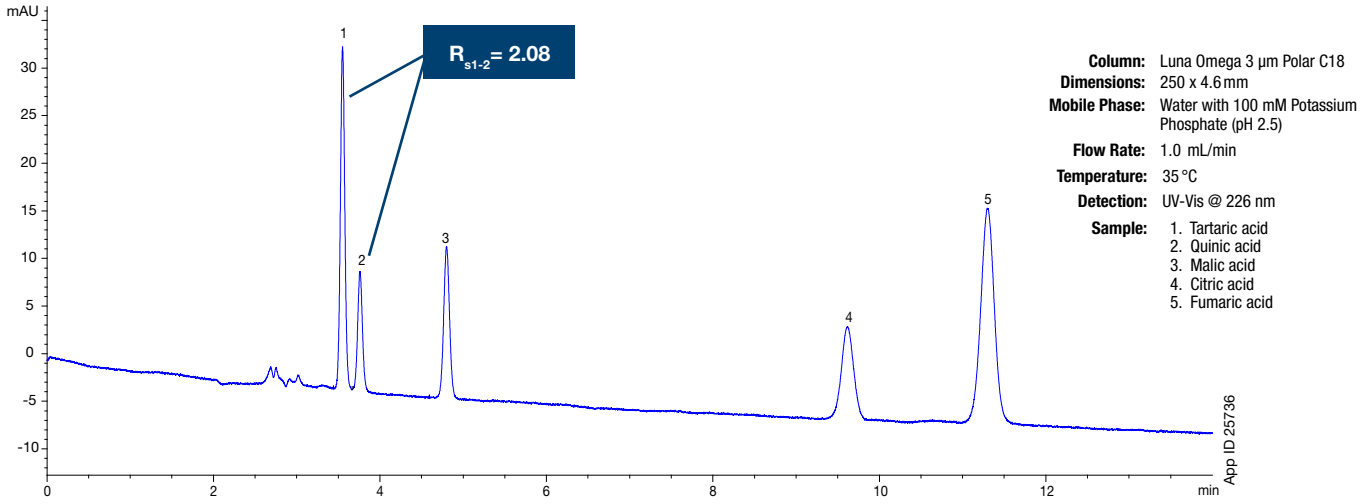




Discussion

Under the above referenced conditions, Tartaric acid and Quinic acid were found to be the critical pair and their overall separation was essential to the success of this application. The initial screening was conducted using 3 µm particles with column dimensions of 150 x 4.6 mm at 1.5 mL/min. Under these conditions, it was found Luna Omega Polar C18 displayed the best resolution of this critical pair with a resolution value of 2.18 between peaks 1 and 2 which are associated with Tartaric and Quinic acid, respectively (**Figure 1**). In addition, peak symmetry with Luna Omega Polar C18 was also found to be better than other chemistries evaluated using these conditions. Core-shell silica is typically regarded as a higher efficiency particle and often results in superior peak performance than its fully porous counterpart, as a result of higher efficiency. In this example, however, the consequence of reduced retention related to the core-shell particles reduced available surface area resulting in early elution of the critical pair and reduced resolution. Therefore, this example serves to highlight the benefit in selecting the most appropriate particle morphology for the application. In this case, the higher surface area of the Luna Omega particle provided improved polar compound retention and selectivity.

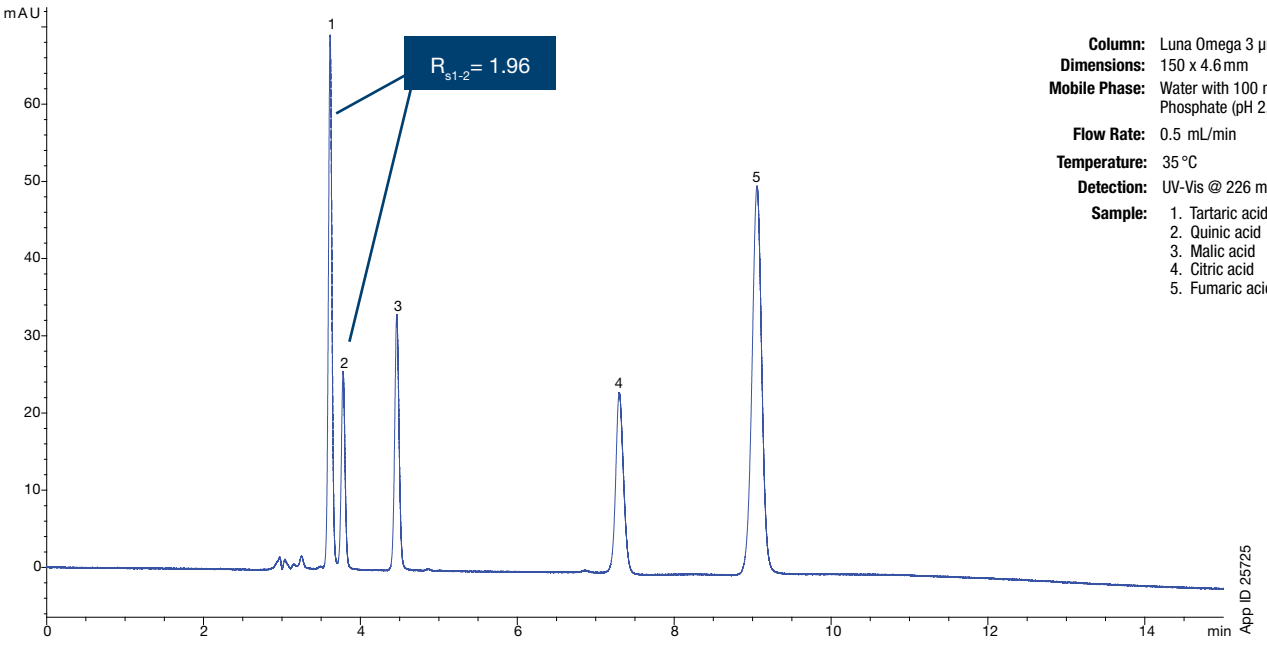
Figure 2.
Luna® Omega 5 µm Polar C18 250 x 4.6 mm @ 1.0 mL/min



Column: Luna Omega 3 µm Polar C18
Dimensions: 250 x 4.6 mm
Mobile Phase: Water with 100 mM Potassium Phosphate (pH 2.5)
Flow Rate: 1.0 mL/min
Temperature: 35 °C
Detection: UV-Vis @ 226 nm
Sample:
1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid

Analyte	RT (min)	Symmetry	Peak Height (mAU)
Tartaric acid	3.55	0.93	36.10
Quinic acid	3.76	0.90	12.60
Malic acid	4.80	0.97	16.00
Citric acid	9.62	1.00	9.80
Fumaric acid	11.30	0.92	22.70

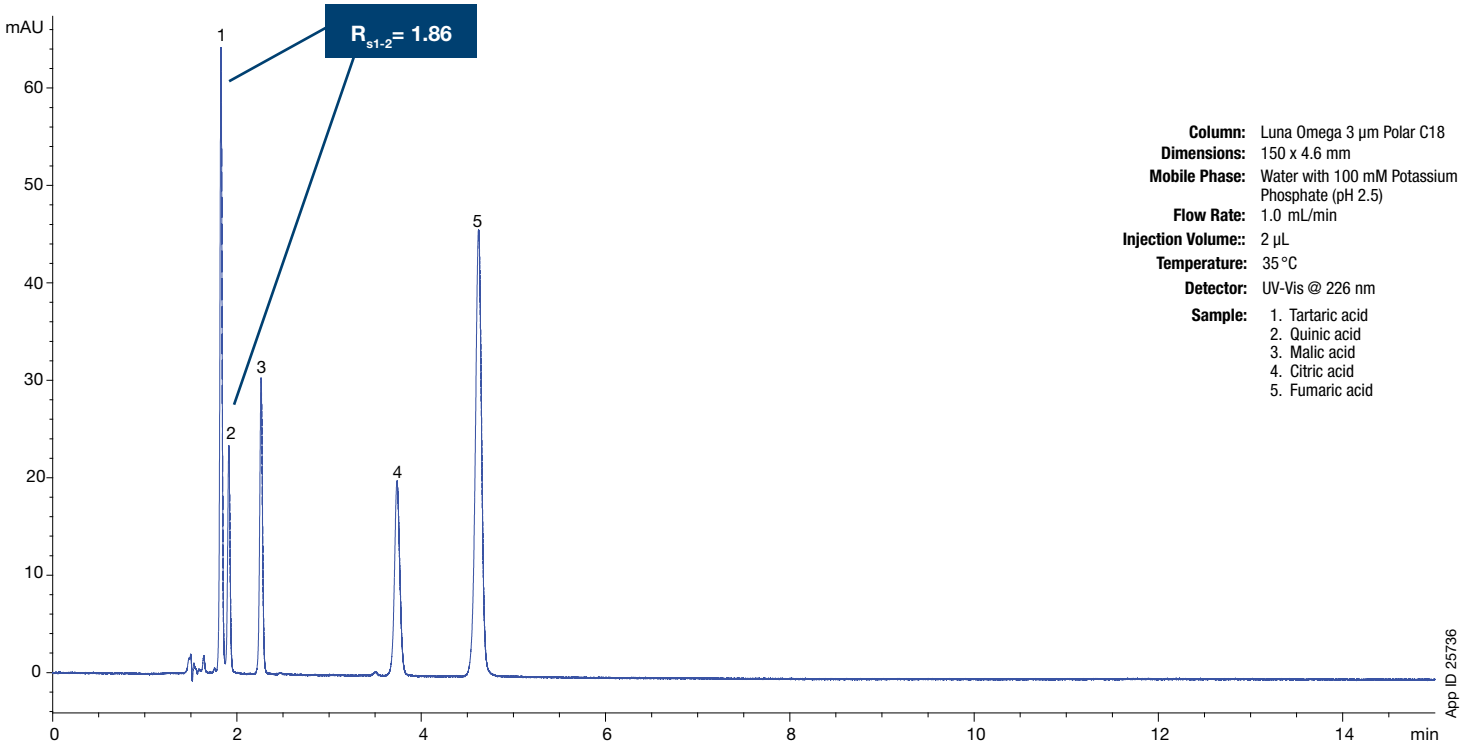
Figure 3.
Luna Omega 3 µm Polar C18 150 x 4.6 mm @ 0.5 mL/min



Column: Luna Omega 3 µm Polar C18
Dimensions: 150 x 4.6 mm
Mobile Phase: Water with 100 mM Potassium Phosphate (pH 2.5)
Flow Rate: 0.5 mL/min
Temperature: 35 °C
Detection: UV-Vis @ 226 mm
Sample:
1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid

Analyte	RT (min)	Symmetry	Peak Height (mAU)
Tartaric acid	3.61	0.952	68.3
Quinic acid	3.78	0.917	25
Malic acid	4.46	0.888	33.1
Citric acid	7.30	0.819	23.4
Fumaric acid	9.06	0.999	50.4

Figure 4.
Luna® Omega 3 µm Polar C18 150 x 4.6 mm @ 1.0 mL/min



Analyte	RT (min)	Symmetry	Peak Height (mAU)
Tartaric acid	1.827	0.969	67.1
Quinic acid	1.912	0.899	22.3
Malic acid	2.262	0.935	30.1
Citric acid	3.737	0.881	20
Fumaric acid	4.622	1.00	45.8

When column length was increased and the flow rate reduced (to maintain constant backpressure), an improvement in the resolution of Tartaric and Quinic acid was seen with a new resolution value of 2.08 (**Figure 2**). This serves to highlight the additional resolving power extra column length brings; the cost, however, is an overall longer run time compared to shorter columns. The run time with a 250 mm column was around 12-minutes, although we found that reducing the column length and particle size we were able to maintain resolution of the critical pair and reduce total analysis time by 25 % (**Figure 3**).

Using a 250 mm column at 1 mL/min backpressure was found to be 97 bar and this was maintained with the 3 µm particle by reducing flow rate to 0.5 mL/min. Under these conditions run time is below 10-minutes and the resolution value was 1.96 between Tartaric and Quinic acid. When the flow rate was doubled on the same column, it still gave a baseline resolution of 1.86 for the critical pair but the observed run time was reduced to under 5-minutes total analysis time (**Figure 4**).

Conclusion

A method using Luna Omega 3 µm Polar C18, a new high efficiency HPLC column with excellent polar selectivity, can be used for the rapid separation of polar organic acids with excellent separation of previously difficult to resolve critical pairs. This technical note shows the benefit of not only screening column chemistries but also particle technologies, such as core-shell and fully porous silica, to optimize the separation of these polar compounds. Here we looked at the effect of run time and column length on the efficiency of the method and their impact on the resolution of these critical pairs.

Kinetex® Ordering Information

2.6 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
Polar C18	00A-4759-AN	00B-4759-AN	00D-4759-AN	00F-4759-AN	AJ0-9532
				for 2.1 mm ID	

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Polar C18	00B-4759-Y0	00D-4759-Y0	00F-4759-Y0	AJ0-9531
			for 3.0 mm ID	

2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Polar C18	00B-4759-E0	00D-4759-E0	00F-4759-E0	AJ0-9530
			for 4.6 mm ID	

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

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Luna® Omega Ordering Information

3 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	AJ0-7600
				for ID:	2.0-3.0 mm

3 µm MidBore Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
			for ID:	2.0-3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
				for ID:	3.2-8.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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