

APPLICATION

Purification of Chlorogenic Acid from Green Coffee using Kinetex® Core-Shell Technology in Axia™ Preparative Formats

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Zeshan loves to collect watches and the Back to the Future Trilogy. He has twin boys which drive him crazy! He is an Apple Fanboy for life and he likes being in the lab more than anywhere else.

Introduction

High performance HPLC/UHPLC core-shell material is the latest technological advancement in chromatographic media. When used under analytical conditions, core-shell particles show improved efficiency and performance over fully porous particles of equivalent particle size.^{1,2}

With the recent commercialization of a lower pressure 5 µm core-shell media, it is now possible to offer core-shell media in a preparative format (>20mm ID) that is compatible with standard prep LC systems.

In this technote, we will demonstrate that this new 5 µm core-shell particle size, available in a variety of bonded phases, can be packed efficiently in preparative formats with internal diameter greater than 2 centimeters. We will highlight the advantage of such product for the isolation of antioxidant **chlorogenic acid** from Green Coffee.

Results and Discussion

Core-shell particles are composed of an inner solid core surrounded by a layer of fully porous silica as shown in **Figure 1**. Those particles were developed more than 50 years ago by C. Horvath¹ and reintroduced in 2009 by Phenomenex due to the advantage they offer over fully porous material

in regards to column efficiency. Axia preparative columns (>20mm ID) were introduced in 2006. The Axia HPLC columns incorporate a patented Hydraulic Piston Compression technology that increases bed density and eliminates media bed collapse as a source of premature column failure. **Figure 2**. Unlike traditional pre-packed preparative column packing methods, the Axia packing method is completely automated and computer monitored which results in improved efficiencies, peak symmetries, and overall column performance.

Figure 1. Core-Shell Particle Technology

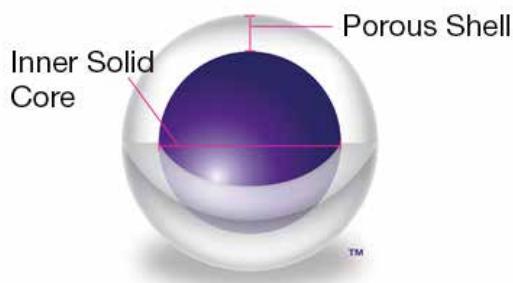
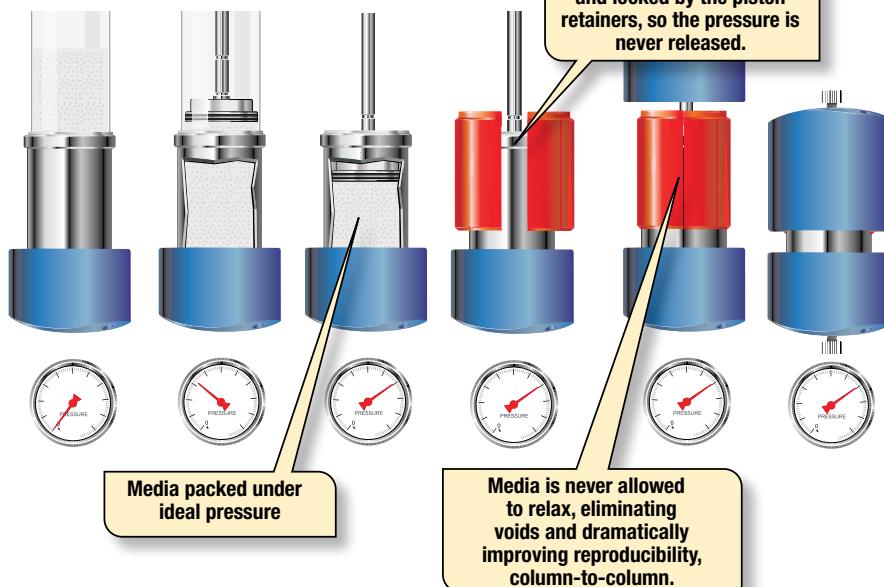


Figure 2. Axia Patented Packing Technology



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Results and Discussion (cont'd)

One investigation performed by F. Gritti and G. Guiochon¹ analyzes the Van Deemter equation for fully porous and core-shell analytical columns and evaluates the contributions of the A, B, and C coefficients to plate height H. They determined that core-shell media significantly reduces band broadening by affecting Eddy dispersion (A), and longitudinal diffusion (B), while mass transfer (C) was determined to be a minor component as depicted in **Figure 3a**. The high column performance is explained essentially by a more homogeneous column packing.² Combining 5 μm core-shell and AxiaTM technology results in efficiency N that can reach up to 125,000 plates/meter for a 21.2 mm ID prep column **Figure 3b**.

The Kinetex core-shell family of particles are designed such that the shell volume to particle size ratio is maintained throughout the particle size. This means that regardless of particle size (1.3, 1.7, 2.6, and 5 μm), the retention and

the selectivity remains constant, allowing for excellent scalability of methods from particle and/or system platforms. Kinetex core-shell 5 μm particles are available in eight (8) different phases for unique selectivities (phases currently available are C18, XB-C18, EVO C18, C8, Phenyl-Hexyl, Biphenyl, PFP and HILIC). Six phases are seen in **Figure 4**.

Prior to the scale-up of preparative conditions, we screened 6 RP core-shell phases to evaluate the selectivity in order to choose the best phase for the isolation of pure **chlorogenic acid** from green coffee extract (the green coffee extract was obtained from ChromaDex[®]). The results using a 20 min gradient are represented in **Figure 4**. Under 0.1 % TFA conditions, the best separation was obtained on a Kinetex 5 μm XB-C18 column. It is interesting to note that with the C8 phase, the selectivity was reversed between **chlorogenic acid** and impurity B.

Figure 3a. Core-Shell Particles Impact on Band Broadening and Column Efficiency

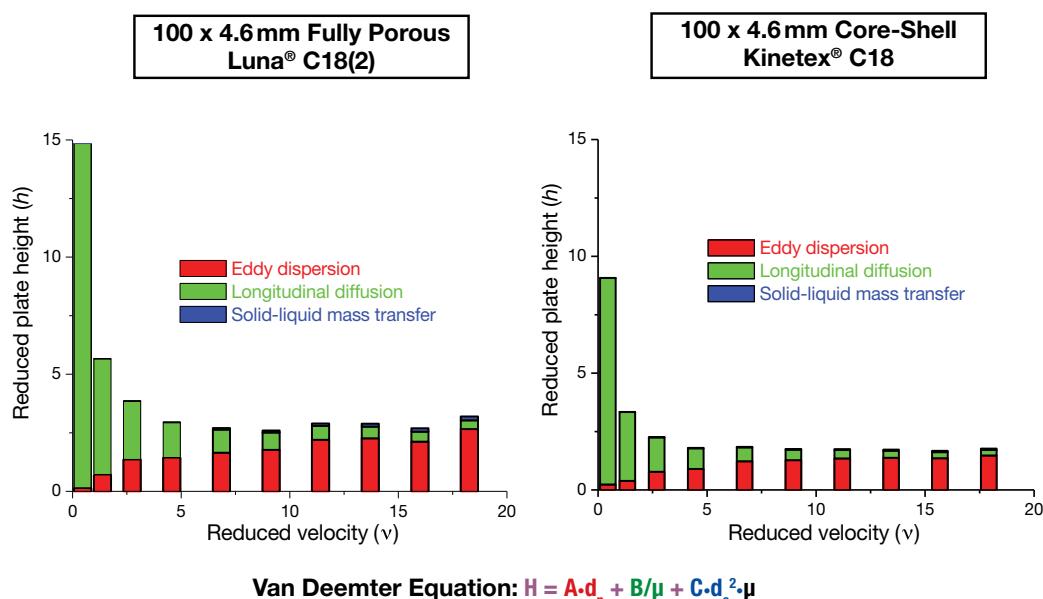
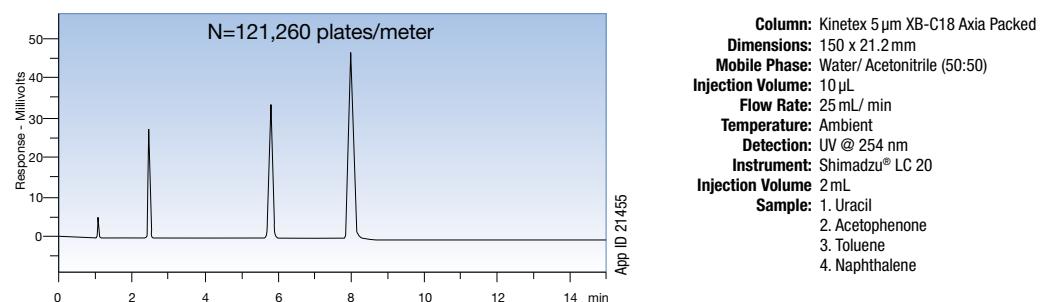
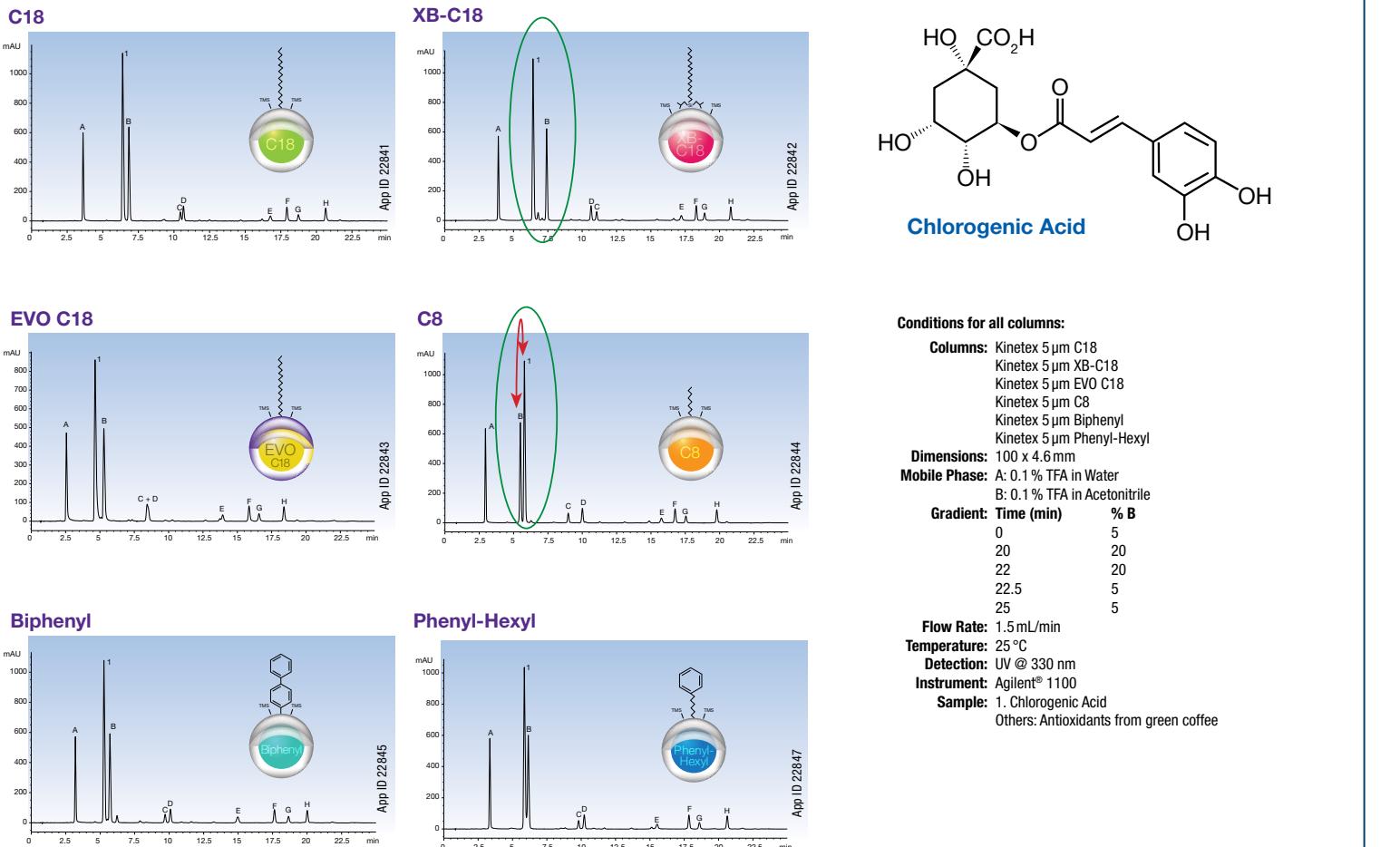


Figure 3b. Naphthalene peak efficiency for a Kinetex 5 μm XB-C18 Axia preparative column



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Figure 4. Screening of Green Coffee Extract on Various Kinetex® Phases



Conditions for all columns:

Columns: Kinetex 5 µm C18

Kinetex 5 µm XB-C18

Kinetex 5 µm EVO C18

Kinetex 5 µm C8

Kinetex 5 µm Biphenyl

Kinetex 5 µm Phenyl-Hexyl

Dimensions: 100 x 4.6 mm

Mobile Phase: A: 0.1% TFA in Water

B: 0.1% TFA in Acetonitrile

Gradient: Time (min) % B

0 5

20 20

22 20

22.5 5

25 5

Flow Rate: 1.5 mL/min

Temperature: 25 °C

Detection: UV @ 330 nm

Instrument: Agilent® 1100

Sample: 1. Chlorogenic Acid

Others: Antioxidants from green coffee

Packing Material	Phase Characteristics					Applications					Type of Compounds			Loading	
	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	pH Range	Small Molecules	Peptides	Proteins	Chiral	Oligonucleotides	Acids	Polar	Hydro-phobic	Bases	Available Surface Area
Kinetex C18	1.3, 1.7, 2.6, 5	100	200	12	1.5-8.5*	●	●				●	●	●	●	●
Kinetex XB-C18	1.7, 2.6, 5	100	200	10	1.5-8.5*	●	●				●	●	●	●	●
Kinetex EVO C18	1.7, 2.6, 5	100	200	11	1-12	●	●				●	●	●	●	●
Kinetex C8	1.7, 2.6, 5	100	200	8	1.5-8.5*	●	●				●	●	●	●	●
Kinetex Phenyl-Hexyl	1.7, 2.6, 5	100	200	11	1.5-8.5*	●	●				●	●	●	●	●
Kinetex Biphenyl	1.7, 2.6, 5	100	200	11	1.5-8.5*	●	●				●	●	●	●	●

Key: ● Best Suited ○ Very Good

*Columns are pH stable from 1.5-10 under isocratic conditions. Columns are pH stable 1.5-8.5 under gradient conditions.



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Results and Discussion (cont'd)

After screening and before purification, we optimized the method and used a Kinetex® 5 µm XB-C18 150 x 4.6 mm ID (Internal diameter) column with a 25 min gradient as depicted in **Figure 5**. We also increased the loading up to 5 mg on-column. (**Figure 6**).

Figure 5. Analysis of Crude Green Coffee Extract

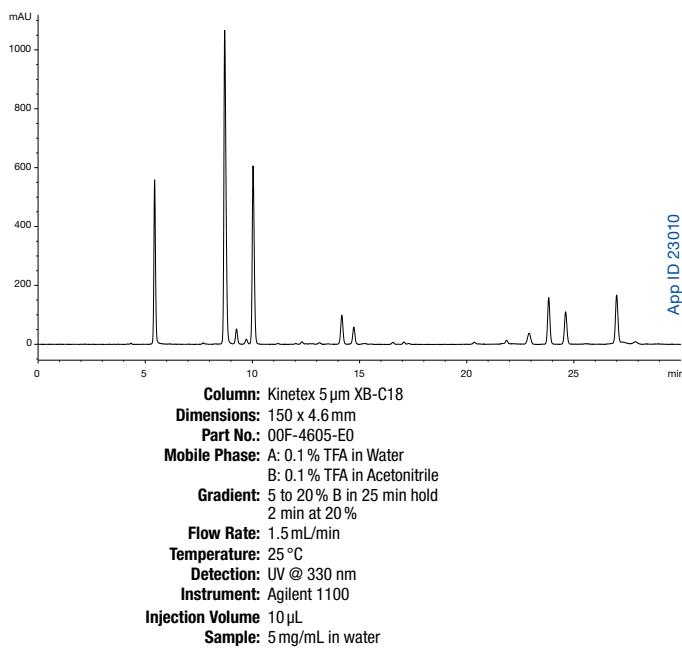
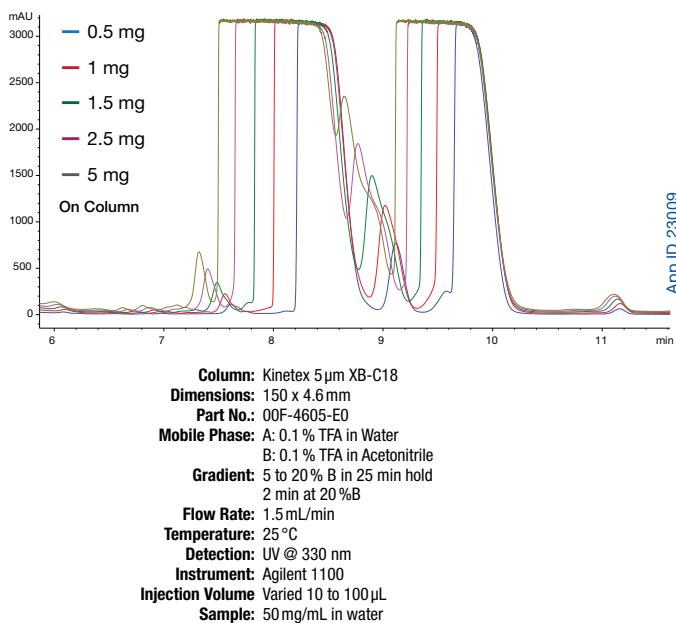
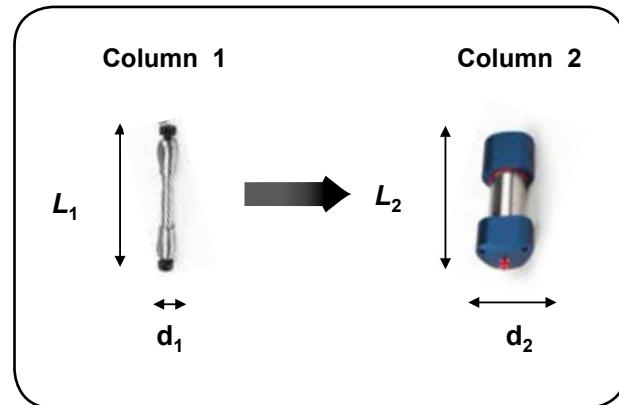


Figure 6. Loading Experiments on Analytical Column



The separation was then scaled-up to a 21.2 mm ID Axia™ preparative column packed with same core-media and the loading was increased accordingly to 100 mg of green coffee extract (scaling factor of 20 from 4.6 to 21.2 mm ID) as represented in **Figure 7**. Fractions of 10 mL were collected, analyzed, and the fractions containing **chlorogenic acid** with purity greater than 94 % at 330 nm, were pooled together (**Fraction 3-5**). Fraction overlays are shown in **Figure 8** and the overlay includes reference standard obtained from ChromaDex, Inc. Analysis of the pool shows a purity greater than 99 % and contained approximately 5 mg of pure product for a recovery of 42 % in one run (**Figure 9**).

Scaling parameter and basic equations



Scaling Factor SF

$$SF = (d_2/d_1)^2$$

Flow Rate F

$$F_2 = F_1 \times SF$$

Loading m

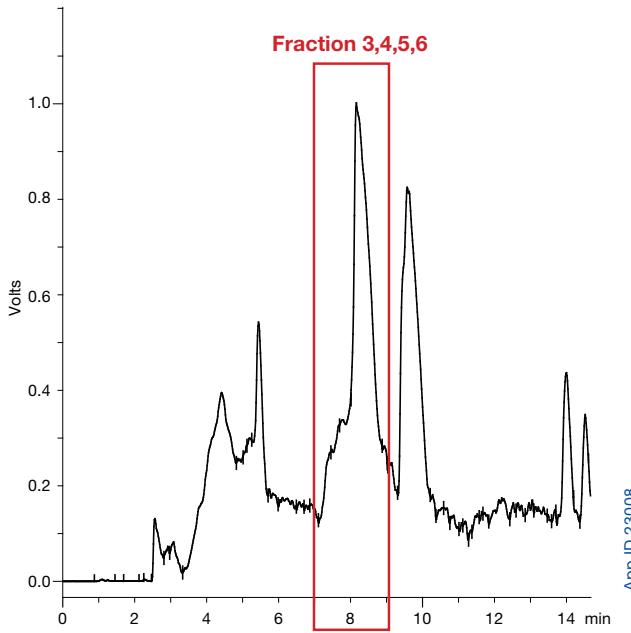
$$m_2 = m_1 \times \frac{L_2}{L_1} \times SF$$

Notes:

- Recommended to keep particle size and length constant
- If column length is increased, loading can be increased proportionally and retention time as well as backpressure will also increase.
- With scale up, change in hardware and flow cell may be required

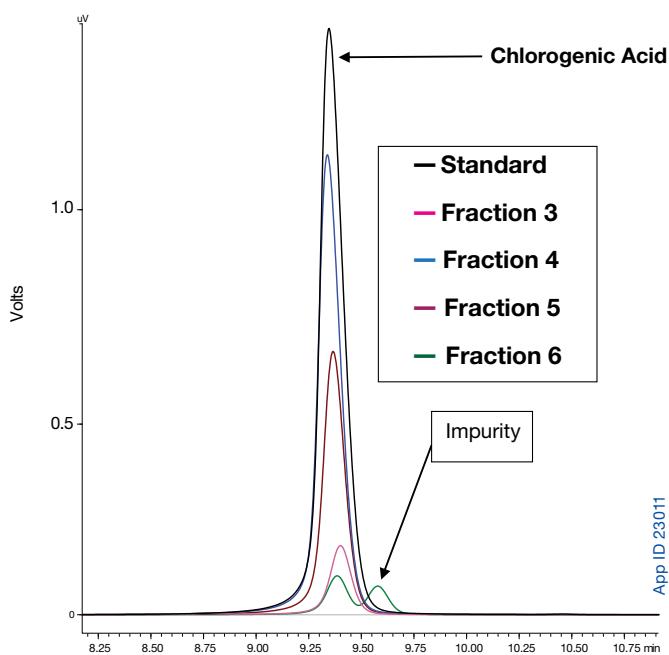
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Figure 7. Preparative Purification of Chlorogenic Acid



Column: Kinetex 5 μ m XB-C18
Dimensions: 150 x 21.2 mm
Part No.: 00F-4605-PO-AX
Mobile Phase: A: 0.1 % TFA in Water
B: 0.1 % TFA in Acetonitrile
Gradient: 5 to 20 % B in 25 min
Flow Rate: 30 mL/min
Temperature: 25 °C
Detection: UV @ 330 nm
Instrument: Shimadzu® LC 20
Injection Volume: 2 mL
Sample: 50 mg/mL in water

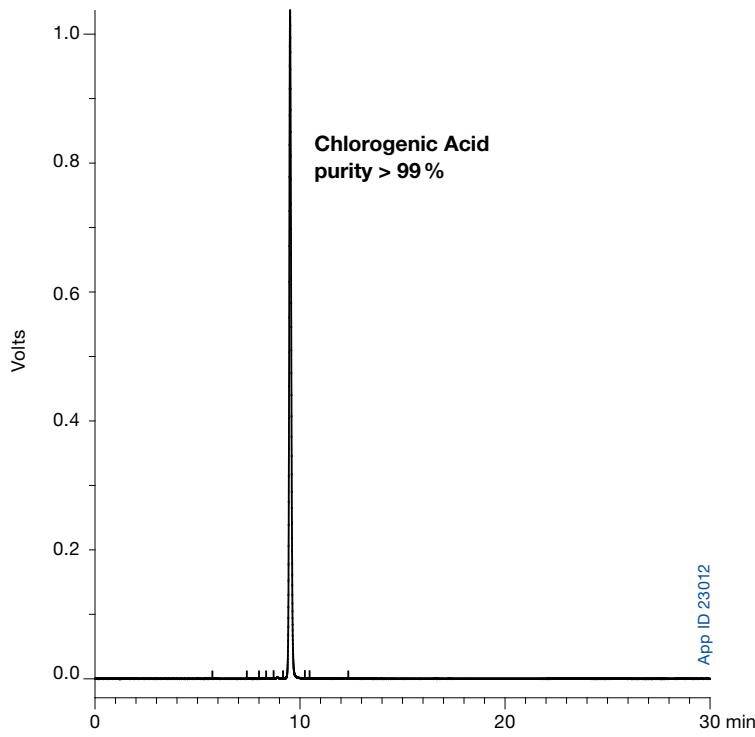
Figure 8. Purification Fractions Overlay



Conditions: Same as figure 5

Instrument: Shimadzu Prominence® LC

Figure 9. Purity for Pooled Fractions



Conditions: Same as figure 5
Instrument: Shimadzu Prominence LC

Conclusion

With the release of Kinetex® core-shell 5 μ m media, it is now possible to perform lab-scale purifications and as a result take advantage of core-shell technology for both analytical and preparative purposes.

Combination of Kinetex core-shell 5 μ m particle and Axia™ patented packing technologies result in preparative columns with high efficiency in the range of 25-60 % greater than columns packed with fully porous media of equivalent particle size.

Scale up from analytical sub-2 μ m to preparative columns in 8 different bonded phases is now feasible and readily available.

From green coffee extract, we were able to recover pure chlorogenic acid with purity of 99 % at 330 nm and a recovery of 40 % in one purification run using an Axia preparative column 150 x 21.2 mm ID. This can be scaled up further to a large format such as 250 x 30 mm ID for increased throughput and yield.

References

1. F. Gritti and G. Guiochon *LC-GC*, **2012**, 30, 586-595.
2. F. Gritti and G. Guiochon *J. Chromatogr. A*, **2013**, 1230, 35-50.



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Kinetex® Ordering Information

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
Biphenyl	00A-4627-AN	00B-4627-AN	00D-4627-AN	—	AJ0-9209
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
C8	—	00B-4608-AN	00D-4608-AN	—	AJ0-8784
Phenyl-Hexyl	—	00B-4603-AN	00D-4603-AN	—	AJ0-8788

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
Biphenyl	00B-4627-Y0	00D-4627-Y0	00F-4627-Y0	AJ0-9208
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
C8	00B-4608-Y0	00D-4608-Y0	—	AJ0-8777
Phenyl-Hexyl	00B-4603-Y0	00D-4603-Y0	—	AJ0-8781

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
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
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
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774

for 4.6 mm ID

5 µm Semi-Preparative Columns (mm)			SecurityGuard SemiPrep Cartridges ^{***}
Phases	150 x 10	250 x 10	10 x 10
C18	00F-4601-N0	00G-4601-N0	AJ0-9278
Biphenyl	00F-4627-N0	00G-4627-N0	AJ0-9280

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
Biphenyl	00B-4627-P0-AX	00D-4627-P0-AX	00F-4627-P0-AX	00G-4627-P0-AX	AJ0-9272
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
C8	00B-4608-P0-AX	00D-4608-P0-AX	00F-4608-P0-AX	00G-4608-P0-AX	AJ0-9205
Phenyl-Hexyl	00B-4603-P0-AX	00D-4603-P0-AX	00F-4603-P0-AX	00G-4603-P0-AX	AJ0-9147
HILIC	—	00D-4606-P0-AX	00F-4606-P0-AX	00G-4606-P0-AX	AJ0-9277

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
Biphenyl	—	—	00F-4627-U0-AX	—	AJ0-9273
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
C8	00B-4608-U0-AX	00D-4608-U0-AX	00F-4608-U0-AX	00G-4608-U0-AX	AJ0-9217
Phenyl-Hexyl	00B-4603-U0-AX	00D-4603-U0-AX	00F-4603-U0-AX	00G-4603-U0-AX	AJ0-9216

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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Kinetex® Ordering Information

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
F5	00A-4723-AN	00B-4723-AN	—	00D-4723-AN	00F-4723-AN	AJ0-9322
Biphenyl	00A-4622-AN	00B-4622-AN	—	00D-4622-AN	00F-4622-AN	AJ0-9209
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
C8	00A-4497-AN	00B-4497-AN	00C-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784
HILIC	00A-4461-AN	00B-4461-AN	00C-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786
Phenyl-Hexyl	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788

for 2.1 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
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
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
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	—	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781

for 3.0 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
F5	—	00B-4723-E0	—	00D-4723-E0	00F-4723-E0	AJ0-9320
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	AJ0-9207
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
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774

for 4.6 mm ID

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	
F5	—	00B-4722-AN	00D-4722-AN	00F-4722-AN	AJ0-9322	
Biphenyl	—	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209	
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	
C8	00A-4499-AN	00B-4499-AN	00D-4499-AN	00F-4499-AN	AJ0-8784	
HILIC	00A-4474-AN	00B-4474-AN	00D-4474-AN	—	AJ0-8786	
Phenyl-Hexyl	—	00B-4500-AN	00D-4500-AN	00F-4500-AN	AJ0-8788	

for 2.1 mm ID

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		
C8	00A-4499-Y0	00B-4499-Y0	00D-4499-Y0	AJ0-8777		
HILIC	—	00B-4474-Y0	—	AJ0-8779		

for 3.0 mm ID

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

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

If Kinetex analytical columns do not provide you with at least equivalent separations to a competing column of the same phase, particle size, and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

For additional technical notes, visit www.phenomenex.com

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