APPLICATIONS

Fast and Sensitive Analysis of PAHs using Kinetex® 3.5 µm PAH Core-Shell LC Columns: EPA Method 8310

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Overview

PAHs (polynuclear aromatic hydrocarbons; also referred to as polycyclic or polyaromatic hydrocarbons) are a class of highly lipophilic environmental pollutants composed of multiple fused aromatic rings. Anthropogenic PAHs are generated through a variety of routes, including incomplete combustion of organic matter (e.g. fossil fuels), industrial processes such as oil refining, and can even be generated by exposing food products to high heat, such as from grilling. Several PAHs have been indicated as possible carcinogens, with benzo[a]pyrene being currently identified as the most dangerous[1]. Because of their potential carcinogenic properties, PAHs are among the most highly monitored environmental contaminants, and standardized methods exist for the analysis of PAHs from a wide variety of sources, including water, soil, air, and food. In EPA Method 8310, The United States Environmental Protection Agency (EPA) provides a list of 18 PAHs to be analyzed from ground water and waste, and provides guidelines for HPLC analyses of these 18 PAHs.

In this Technical Note, we provide analytical LC methods for the chromatographic separation of the 18 PAHs outlined in EPA Method 8310 using a simple water and acetonitrile gradient and a new core-shell based column for PAH analysis – Kinetex® 3.5 µm PAH. Columns packed with core-shell media provide significantly improved efficiency than columns packed with traditional fully porous particles, resulting in improved resolution between closely-eluting peaks and increased peak height response and sensitivity. In addition, because of the relatively low surface area of core-shell media, total analysis times are generally much shorter, increasing productivity and reducing waste generation and solvent consumption. Please note that, while EPA Method 8310 calls for detection via UV and fluorescence, the chromatograms in this technical note were generated solely using UV detection and were meant to demonstrate chromatographic performance for the separation of these challenging molecules.

Analyte List	
1	Naphthalene
2	Acenaphthylene
3	1-Methylnaphthalene
4	2-Methylnaphthalene
5	Acenaphthene
6	Fluorene
7	Phenanthrene
8	Anthracene
9	Fluoranthene
10	Pyrene
11	Benz[a]anthracene
12	Chrysene
13	Benzo[b]fluoranthene
14	Benzo[k]fluoranthene
15	Benzo[a]pyrene
16	Dibenz[a,h]anthracene
17	Benzo[g,h,i]perylene
18	Indeno[1,2,3-cd]pyrene

LC Conditions

Figure 1.

Column: Waters® 5 µm PAH C18
Dimension: 250 x 4.6 mm
Mobile Phase: A: Water
B: Acetonitrile

Gradient	Time (min)	% B
	0	60
	17.5	100
	25	100

Flow Rate: 1.2 mL/min
Backpressure: 160 Bar
Temperature: 35 °C
Detection: UV @ 292 nm

Figure 2.

Column: Kinetex 3.5 µm PAH
Dimension: 250 x 4.6 mm
Part No.: 00G-4764-E0
Mobile Phase: A: Water
B: Acetonitrile

Gradient	Time (min)	% B
	0	60
	17.5	100
	25	100

Flow Rate: 1.2 mL/min
Backpressure: 230 Bar
Temperature: 35 °C
Detection: UV @ 292 nm

Figure 3.

Column: Kinetex 3.5 µm PAH
Dimension: 100 x 4.6 mm
Part No.: 00D-4764-E0
Mobile Phase: A: Water
B: Acetonitrile

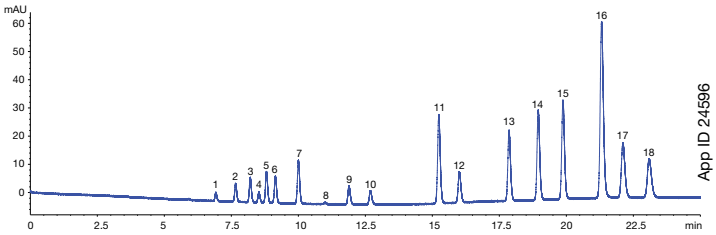
Gradient	Time (min)	% B
	0	50
	7	100

Flow Rate: 1.2 mL/min
Backpressure: 160 Bar
Temperature: 35 °C
Detection: UV @ 292 nm

Results

Kinetex® 3.5 µm PAH is the first core-shell based column made specifically for the analysis of PAHs, bringing the efficiency benefits of core-shell morphology to the field of PAH analysis. **Figure 1** is a representative chromatogram obtained using a fully porous column marketed specifically for PAH analysis (Waters® 5 µm PAH C18, 250 x 4.6 mm), the gradient and flow rate were reproduced from the manufacturers product marketing literature. Using that column and running conditions, you can see that all 18 analytes in EPA Method 8310 are baseline resolved, with a total cycle time of ~28 minutes with re-equilibration. Under these conditions, the lowest resolution is obtained between peaks 4 and 5, 2-methylnaphthalene and acenaphthene, with an Rs of 1.92.

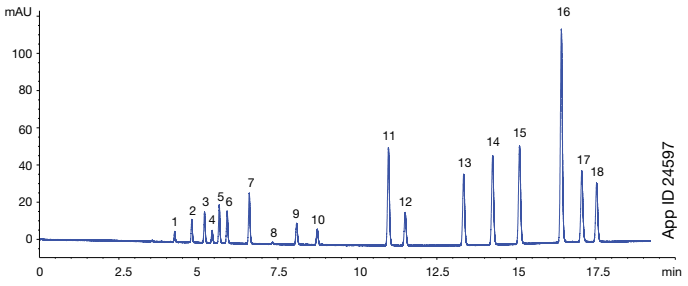
Figure 1. Separation of the 18 EPA Method 8310 PAHs using a fully porous Waters 5 µm PAH C18 250 x 4.6 mm column.



When run using the identical running conditions, the Kinetex 3.5 µm PAH, 250 x 4.6 mm core-shell column has a total cycle time of about 20 minutes (with re-equilibration), which is already about 25 % faster than the fully porous Waters 5 µm PAH C18 250 x 4.6 mm column. Using the Kinetex 3.5 µm PAH column, the minimum resolution value occurs between the same two peaks, 2-methylnaphthalene and acenaphthene, with an Rs of 2.86, which is 48 % better than the resolution for those peaks on the Waters 5 µm PAH C18 250 x 4.6 mm column (Rs = 1.92).

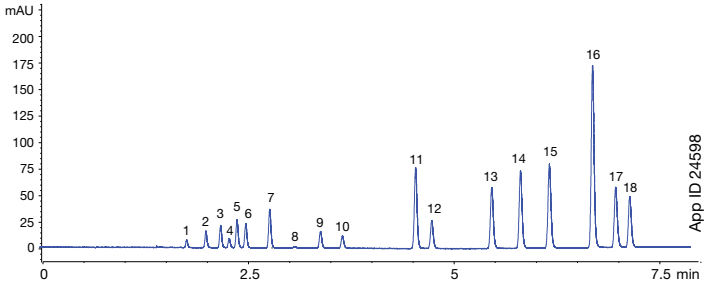
Thus, by simply moving from the fully porous Waters 5 µm PAH C18 250 x 4.6 mm column to the Kinetex 3.5 µm PAH 250 x 4.6 mm core-shell column under identical conditions, we increased our resolution value by 48 % and also reduced our analysis time by ~25 %. This is possible because of the high efficiency advantage of the Kinetex core-shell particle morphology, and is further enhanced by the slightly smaller particle diameter

Figure 2. Separation of the 18 EPA Method 8310 PAHs using a core-shell Kinetex 3.5 µm PAH 250 x 4.6 mm column (same running conditions as **Figure 1**)



With the additional resolution provided by the Kinetex 3.5 µm PAH core-shell media, we can now sacrifice a little efficiency by using a shorter column. This will allow us to further reduce our analysis times. This is shown in **Figure 3**, where we have moved to a 100 x 4.6 mm Kinetex 3.5 µm PAH column and a shorter gradient profile. Under these new conditions, and with this shorter column format, our total cycle time is about 9 minutes, which is about 1/3 of the original method. That is almost a 3-fold increase in productivity. Under these conditions, the minimum Rs value is 1.97 between 2-methylnaphthalene and acenaphthene, which is still better than the original resolution value obtained using the starting method on the Waters 5 µm PAH C18 250 x 4.6 mm column.

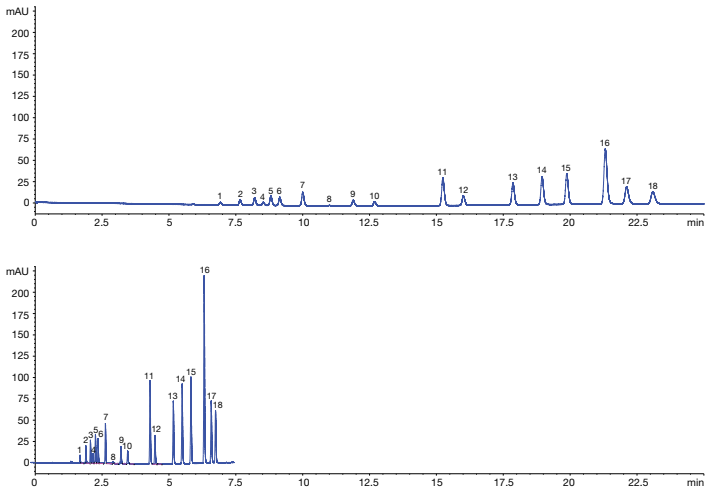
Figure 3. Accelerated analysis of the EPA Method 8310 PAH mixture using a 100 x 4.6 mm column packed with Kinetex 3.5 µm PAH core-shell media.



The chromatograms in **Figures 1-3** are shown in the same time scale so that you can clearly see how the run time is decreased. In **Figure 4**, we have overlaid the original chromatogram obtained using the Waters 5 µm PAH C18 250 x 4.6 mm and the Kinetex 3.5 µm PAH 100 x 4.6 mm column, but adjusted the x-axis time scale so that you can better compare the resolution on the 9 minute Kinetex method to the 28 minute original method. You can clearly see how well the resolution has been preserved even though the run time is only about 1/3 as long as the original method. Also note the increase in peak height that is achieved using the Kinetex 3.5 µm PAH column. For instance, the peak height response for Dibenz[a,h]anthracene, the largest peak, is about 80 mAU using the Waters 5 µm PAH C18 250 x 4.6 mm column. But that same sample injection yields a peak height response of ~220 mAU on the Kinetex 3.5 µm PAH 100 x 4.6 mm column, almost a three-fold increase in sensitivity.

Comparative separations may not be representative of all applications.

Figure 4. Comparison of the original method obtained using the Waters® 5 µm PAH C18 250 x 4.6 mm (**TOP**) with the same sample analyzed using the 100 x 4.6 mm column packed with Kinetex® 3.5 µm PAH core-shell media (**BOTTOM**).



Comparative separations may not be representative of all applications.

Conclusions

The new Kinetex 3.5 µm PAH introduces the core-shell efficiency advantage to the field of PAH analysis. The ultra-high efficiency of the core-shell morphology can provide the analyst with the ability to dramatically improve their resolving power and their productivity. In this case, baseline resolution of the 18 component PAH mixture specified in EPA Method 8310 is achieved with a total cycle time of less than 10 minutes, compared to about 30 minutes using a typical fully porous alternative column (Waters 5 µm PAH C18).

References

1. Kuo et al. 1998; Wang et al. 2002

Kinetex® Ordering Information

3.5 µm Minibore Columns (mm)				SecurityGuard™ULTRA Cartridges (mm)
Phase	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PAH	00B-4764-AN	00D-4764-AN	00F-4764-AN	AJ0-9535 for 2.1 mm ID

3.5 µm MidBore™ (mm)		SecurityGuard ULTRA Cartridges (mm)
Phase	100 x 3.0	3/pk
PAH	00D-4764-Y0	AJ0-9534 for 3.0 mm ID

3.5 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges (mm)
Phase	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
PAH	00D-4764-E0	00F-4764-E0	00G-4764-E0	AJ0-9533 for 4.6 mm ID

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

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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