# Part I: PAH Analysis and EPA Method 8310

Guest Author: Dr. Jeff Layne

Welcome to our newest LC article series! I will be focusing on Phenomenex's latest HPLC/UHPLC product, made specifically for the analysis of PAHs (polynuclear aromatic hydrocarbons) also referred to as **polycyclic or polyaromatic hydrocarbons**. These are a class of highly lipophilic environmental pollutants composed of multiple fused aromatic rings. PAHs can arise from natural sources, but 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 even generated by exposing food products to high heat, such as grilling. Yep—those delicious grill marks on your steak or hamburger contain PAHs—but that's not going to stop me from eating them!

But why do we care about PAHs? Why do we need to test for them? Well, several PAHs have been indicated as possible carcinogens with benzo[a]pyrene, which is identified as one of the most dangerous carcinogenic substances (Kuo et al. 1998; Wang et al. 2002). Due to the potential cancer-causing 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. For instance, in **Method 8310**, the United States Environmental Protection Agency (**EPA**) provides a list of 18 PAHs to be analysed from ground water and waste, and provides guidelines for HPLC analyses of these 18 PAHs.

Now, as you might guess, PAHs can be tricky little beasts to resolve from one another because they are structurally similar and many are isomers. Traditional, monomerically bonded C18 phases lack the shape-selectivity required to resolve complex PAH mixtures,

and it is pretty much mandatory to have a polymerically-bonded C18 in order to effectively analyse these types of molecules.

What do I mean by "polymeric"? Well, in this usage, polymeric refers to a type of stationary phase bonding reaction in which multiple cycles of bonding are carried out such that the C18 ligands can be bonded to one another, as well as being bonded to the underlying silica particle. The net effect is to create a complex matrix of C18 ligands, as opposed to typical single layer monomeric bonding.

It is the polymeric C18 matrix that allows the phase to separate these bulky molecules by the shape, in addition to traditional hydrophobic mechanisms. Now, when we combine that polymeric C18 bonding process with the efficiency advantage of core-shell media, you get a one-two punch with the right selectivity (from the polymeric bonding) and the best efficiency (from the core-shell particle morphology) to provide the optimal column for the fast and effective analysis of PAHs.

**Now I am going to show you a fast and effective LC method for the 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**. Please note that, while EPA Method 8310 calls for detection via UV and fluorescence, the chromatograms in this article were generated solely using UV detection and were meant to demonstrate chromatographic performance for the separation of these challenging molecules.

Kinetex 3.5 µm PAH is the first core-shell based column made specifically for the analysis of PAHs, and brings 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 (Brand X 5 μm PAH, 250 x 4.6mm), the gradient, and the flow rate were reproduced from the manufacturers product marketing literature. Using that column and running conditions, you can see that all 18 analytes in the EPA 8310 are baseline resolved, with a total cycle time of about 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 PAHs in EPA 8310 mixture using a fully porous 250 x 4.6mm column packed with conventional fully porous 5 μm media (Brand X 5 μm PAH 250 x 4.6mm), using running conditions (gradient profile and flow rate) recommended by the manufacturer.
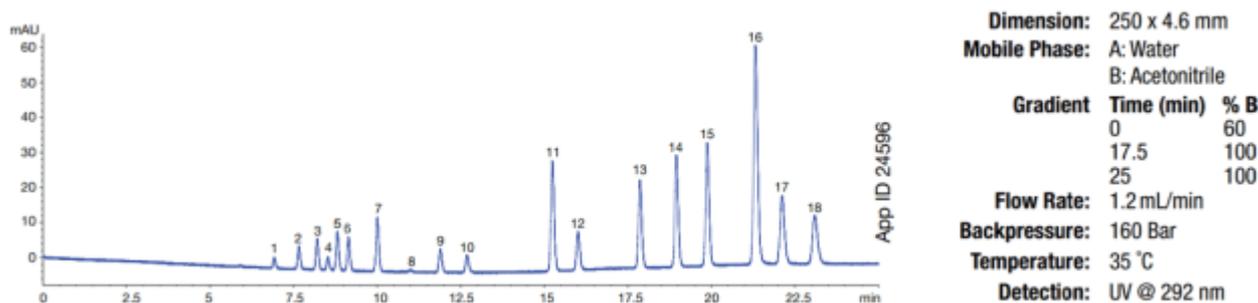


When the method is run using the identical running conditions, the core-shell Kinetex 3.5 μm PAH, 250 x 4.6mm column has a total cycle time of about 21 minutes (with re-equilibration), which is already about 25% faster than the fully porous Brand X 5 μm PAH 250 x 4.6mm column.  Using the Kinetex 3.5 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 Brand X 5 μm PAH C18 250 x 4.6 mm column (Rs = 1.92).

Thus, by simply moving from the fully porous Brand X 5 μm PAH 250 x 4.6mm column to the

core-shell Kinetex 3.5 µm PAH, 250 x 4.6mm column under identical conditions, we are able to increase our resolution value by 48% and also reduce our analysis time by ~25%. This is possible because of the high efficiency advantage of the Kinetex core-shell particle morphology and the slightly smaller particle diameter.

**Figure 2.** Analysis of the same 18 PAHs in EPA 8310 performed using a 250 x 4.6mm column packed with core-shell Kinetex 3.5 µm PAH media, using the identical running conditions.
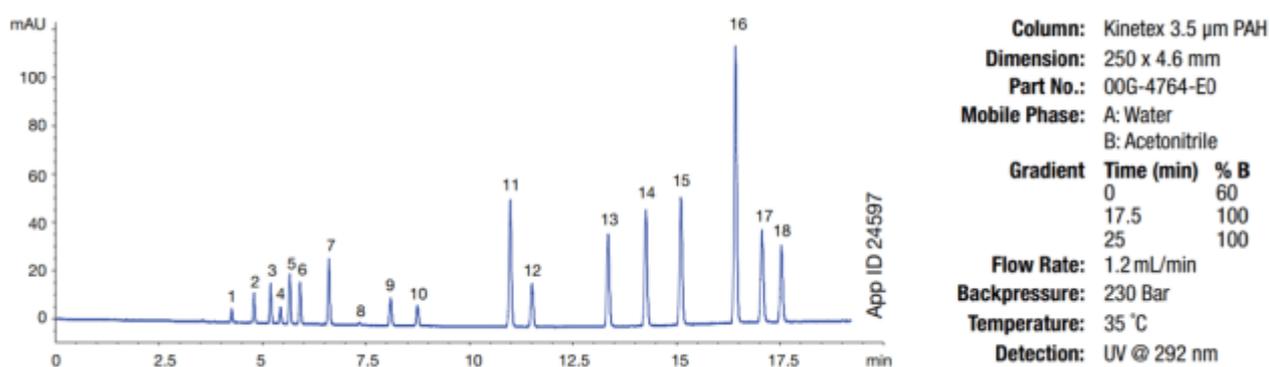


With the additional resolution provided by the Kinetex 3.5 µm PAH, we now sacrifice a little efficiency by using a shorter column. This will allow us to further reduce our analysis times. This is shown in **Figure 3**, where we have moved to a 100 x 4.6mm Kinetex 3.5 µm PAH column and a shorter gradient 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 almost as much as we started with using the original 30-minute method.

**Figure 3.** Accelerated analysis of the EPA8310 PAH mixture using a 100 x 4.6mm column packed with Kinetex

3.5um PAH media.
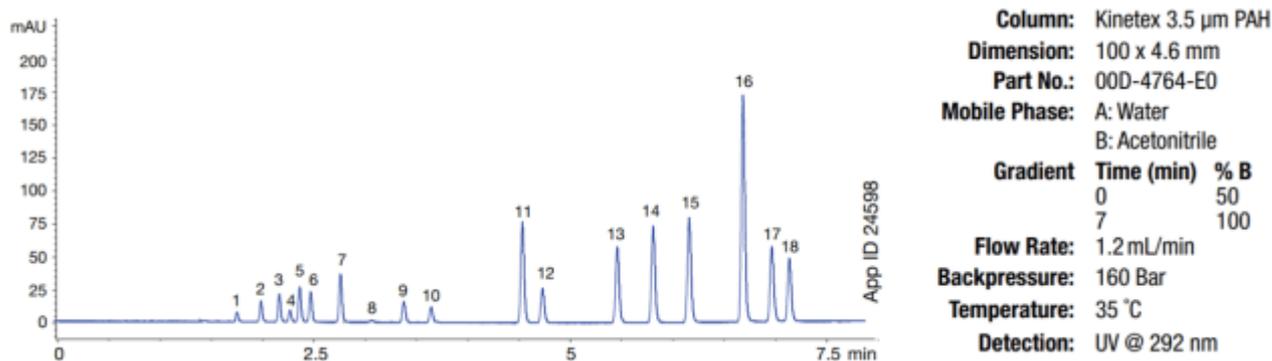


The chromatograms in **Figures 1-3** are shown in the same time scale so that you can clearly see how the run time is decreased.  In **Figure 4**, we have overlaid the original chromatogram obtained using the Brand X 5 µm PAH 250 x 4.6mm and the Kinetex 3.5um PAH 100 x 4.6mm column, but adjusted the 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 ibenz[a,h]anthracene, the largest peak, is about 80mAU using the Brand X 5 µm PAH 250 x 4.6mm column.  But that same sample injection yields a peak height response of ~220mAU on the Kinetex 3.5um PAH 100×4.6mm column, almost a three-fold increase in sensitivity.

**Figure 4.** Comparison of the original method obtained using the Brand X 5 µm PAH 250 x 4.6mm (TOP) with the same sample analyzed using the 100 x 4.6mm column packed with Kinetex 3.5 µm PAH media (BOTTOM).
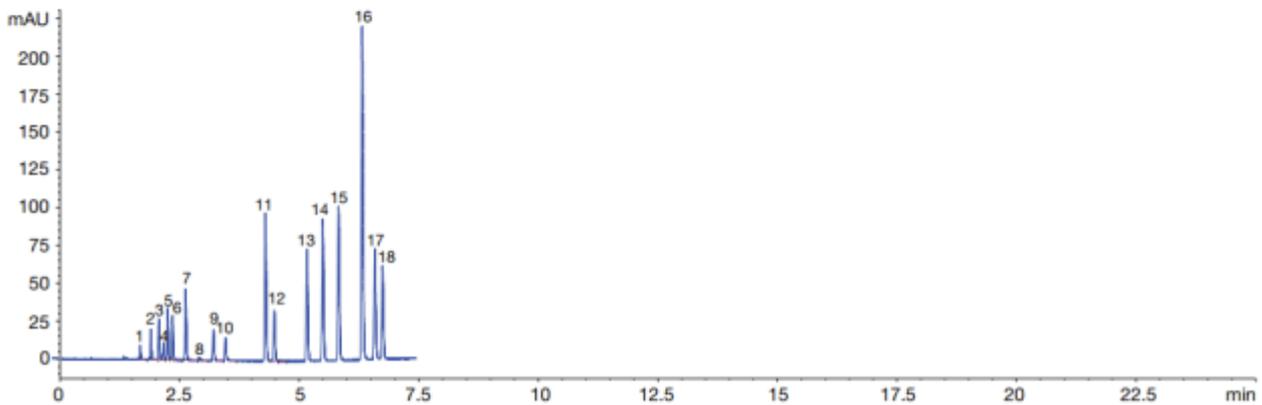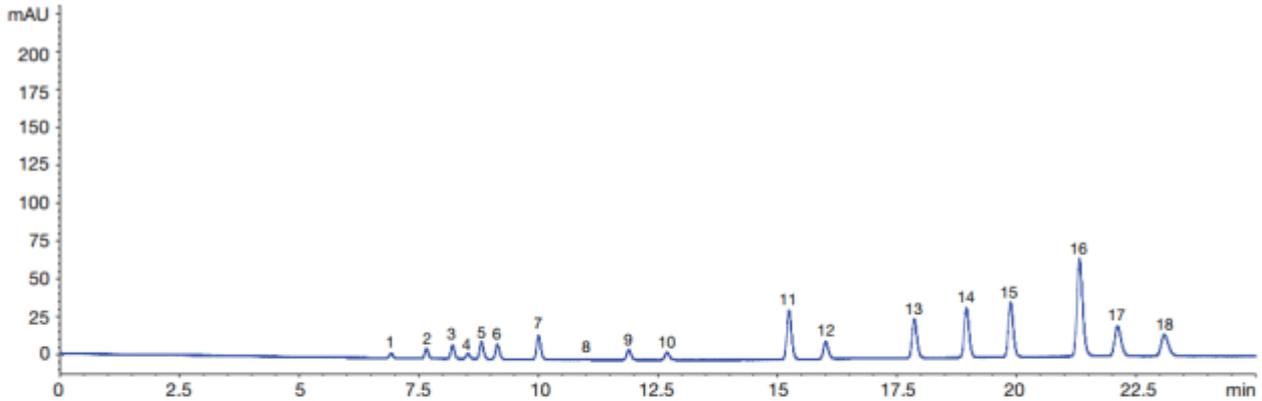
The new Kinetex 3.5 µm PAH brings the core-shell efficiency advantage to the field of PAH analysis. The ultra-high efficiency of the core-shell morphology can provide the analyst with the ability to dramatically improve their resolving power and their productivity.  In this particular case, baseline 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 (Brand X 5 µm PAH).

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| Summary |
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| Article Name |
| Bringing the Performance Advantage of Core-Shell Technology to PAH Analysis-Part 1 |
| Description |
| Dr. Jeff Layne analyzes the performance advantage of core-shell technology used with PAH analysis, starting with EPA Method 8310. |
| Author |
| Dr. Jeff Layne |