# Part 3: **EU 15+1 PAH Method**

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Welcome to the third and last part of my article series on PAH method analysis. In the previous two articles, I have focused on two United States EPA methods for PAH analysis—EPA Method 610 and EPA Method 8310. We now turn our attention a third distinct PAH analytical method, developed by the European Food Safety Authority (EFSA), who have provided a list of 16 priority PAHs to be tested from food products, known as the EU 15+1 PAH mix.

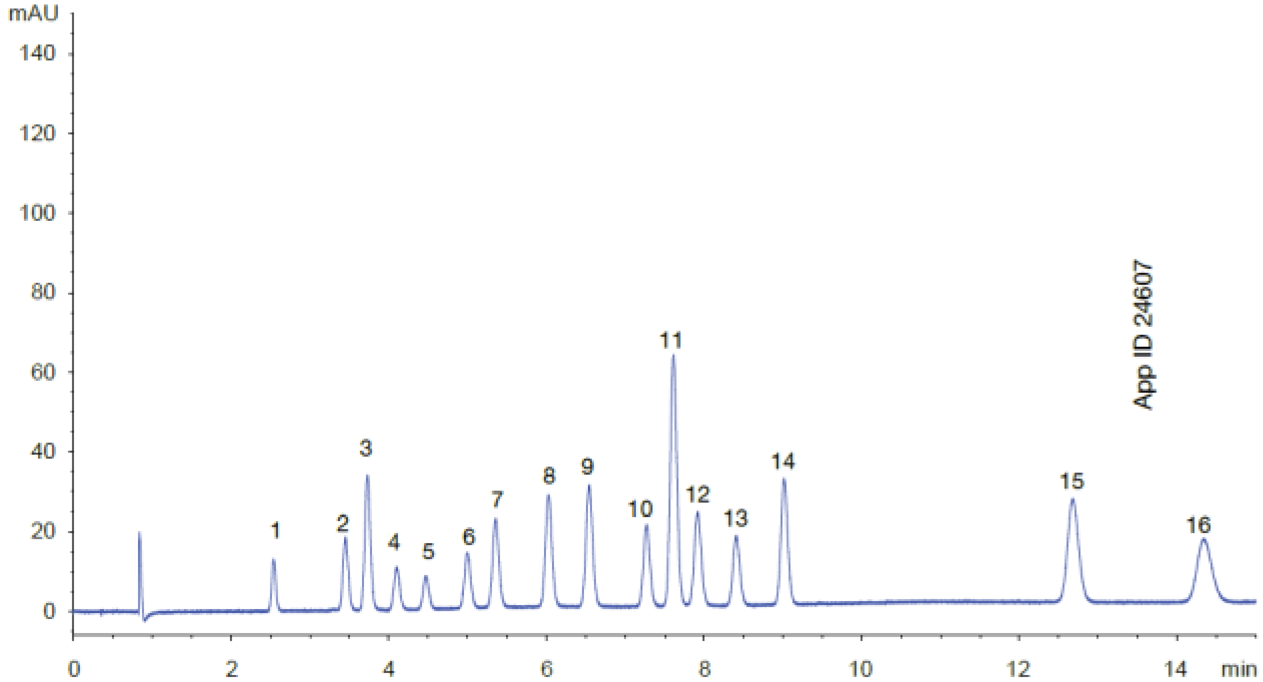
Whereas both of the EPA methods had quite a lot of overlap in their sample composition, the EU 15+1 mixture is quite distinct (see **Table 1** below) and contains two especially problematic analytes that are VERY strongly retained under reversed-phase conditions on a C18 phase—dibenzo(a,i)pyrene and dibenzo(a,h)pyrene. These analytes are very hydrophobic and in this method, we have added a small amount of a very strong RP solvent (THF) to our strong mobile phase B in order to facilitate their elution in a reasonable amount of time. Our goal was to provide better than baseline resolution for all the components in the EU 15+1 mixture, and we could do so with a total cycle time of less than 15 minutes. While it would certainly be possible to further reduce cycle time by using: (a) a shorter column (e.g. 100 x 4.6mm), (b) increasing flow rates, or (c) tweaking the gradient, we leave that up to the discretion of the analysts as certainly those adjustments would result in some loss of resolution.

**Table 1.** EU 15+1 PAH sample mixture.

| # | Name |
|---|---|
| 1 | Benzo[c]fluorene |
| 2 | Cyclopenta[cd]pyrene |
| 3 | Benzo[a]anthracene |
| 4 | Chrysene |
| 5 | 5-Methylchrysene |
| 6 | Benzo(j)fluoranthene |
| 7 | Benzo[b]fluoranthene |
| 8 | Benzo[k]fluoranthene |
| 9 | Benzo[a]pyrene |
| 10 | Dibenzo[a,l]pyrene |
| 11 | Dibenz[a,h]anthracene |
| 12 | Benzo[ghi]perylene |
| 13 | Indeno[1,2,3-cd]pyrene |
| 14 | Dibenzo[a,e]pyrene |
| 15 | Dibenzo(a,i)pyrene |
| 16 | Dibenzo(a,h)pyrene |

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 method analysis. **Figure 1** is a representative chromatogram obtained for the separation of the Eu 15+1 PAH mixture using a fully-porous column marketed specifically for PAH analysis using the gradient and flow rate from the manufacturer's product marketing literature. Using that column and running conditions, you can see that all 16 analytes in the Eu 15+1 mix are well-resolved, with a total cycle time of about 18 minutes with re-equilibration. Under these conditions, the lowest resolution is obtained between peaks 11 and 12, dibenz[a,h]anthracene and benzo[ghi]perylene, with an Rs of 1.88. Note that one of the challenges of this particular mixture is that the last two peaks are extremely hydrophobic and strongly retained in C18 phases. To overcome this, the mobile phase contains a small fraction of THF in mobile phase B (95:5 acetonitrile:THF).

**Figure 1.** Separation of the 16 PAHs in Eu 15+1 mixture using a fully-porous 150 x 3.0mm column packed with conventional fully porous 4 µm media using running conditions (gradient profile and flow rate) recommended by the manufacturer, including the addition of THF to mobile phase B. App. ID. 24607.




Under the running conditions above, taken from the manufacturer's marketing literature, the maximum pressure was in the range of 265 Bar (~3850 PSI). To provide analysts with a lower-pressure alternative format, we ran the exact same sample and running conditions using our core-shell based Kinetex 3.5 µm PAH column in a 150 x 4.6mm format (**Figure 2**). Total cycle time was reduced from about an 18 min cycle time on the fully-porous 4 µm PAH column to about 15 minutes, saving about 3 minutes per injection cycle (~ 16.7%). Although the peak elution order was identical between the two columns, the peaks obtained using the Kinetex 3.5 µm PAH column are significantly narrower and give a greater peak height response. For instance, peak 5 (5-methylchrysene) gave a peak height response of 8.5 mAU on the fully porous 4 µm PAH column but gave a peak height response of 14.8

mAU on the Kinetex 3.5 µm PAH column, a 72% increase in peak height response for the same sample injection (**Figure 3**). In addition, the narrower peaks provide significantly better resolution, and the minimal resolution obtained under these conditions occurred between peaks 10 and 11 (dibenzo[a,l]pyrene and dibenz[a,h]anthracene), with an Rs value of 2.70.

**Figure 2.** Analysis of the same 16 PAHs in Eu 15+1 mixture performed using a 150 x 4.6mm column packed with core-shell Kinetex 3.5 µm PAH media using the same gradient profile as in **Figure 1.** App. ID. 24606.

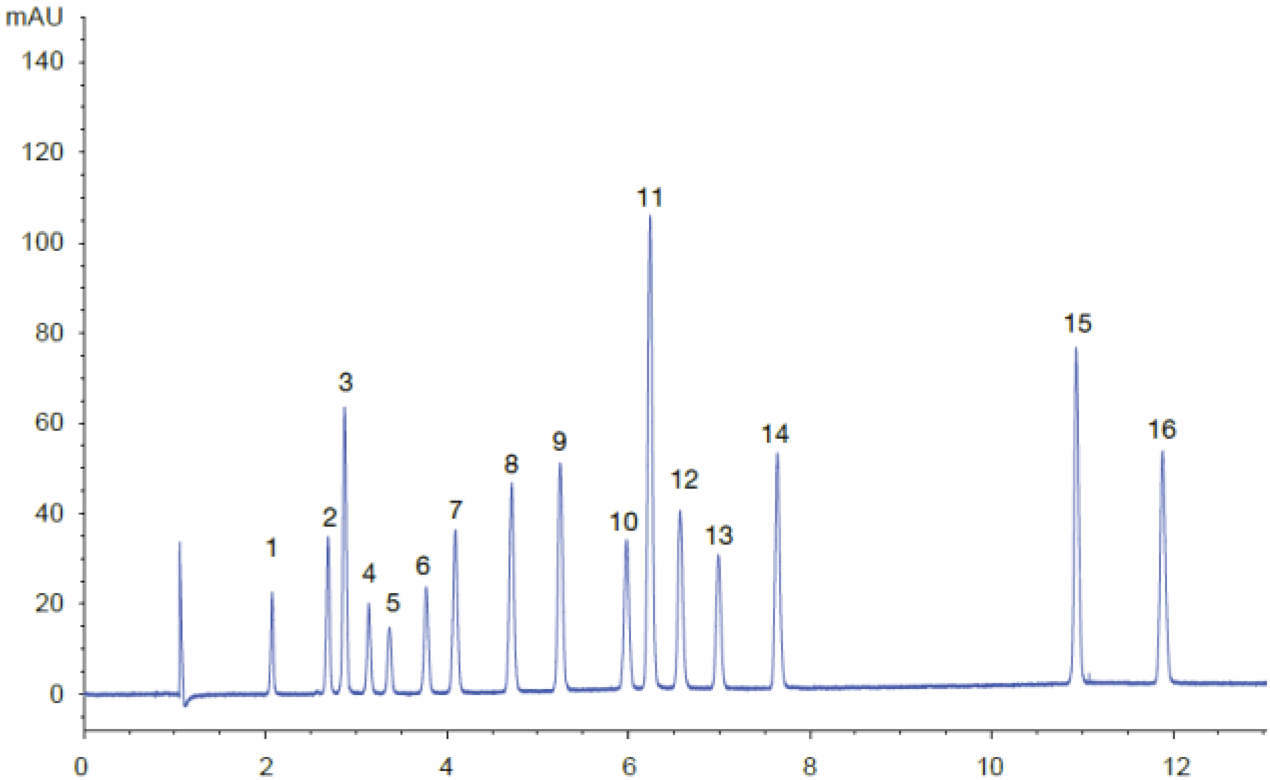
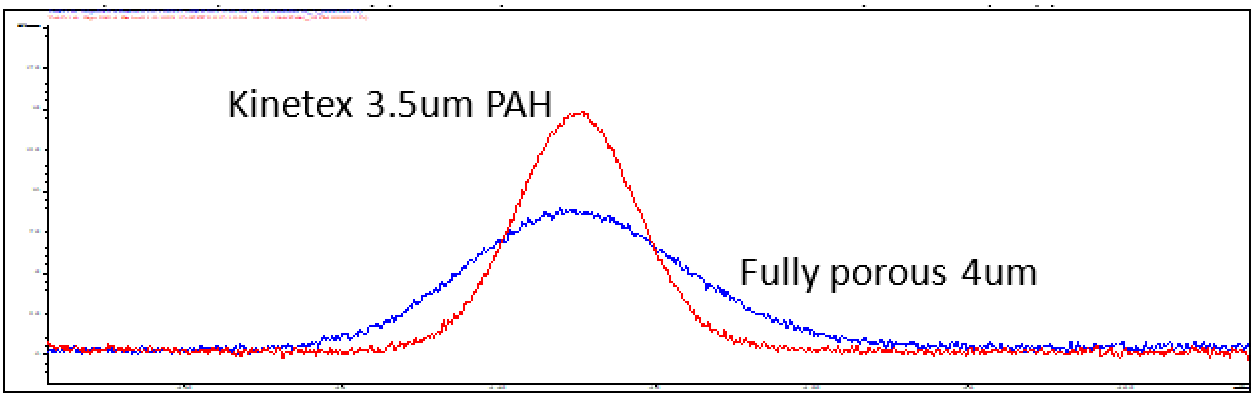



**Figure 3.** Overlay of the peak for 5-methylchrysene obtained using the Kinetex 3.5 µm PAH 150 x 4.6mm column (red trace) and the fully-porous 4 µm PAH 150 x 3.0mm column (blue trace). App. ID. 24607.




The new Kinetex 3.5um PAH bring the core-shell efficiency advantage to the field of PAH method 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 16 component PAH mixture specified in Eu 15+1 PAH mixture is achieved with a total cycle time of about 15 minutes, with more than baseline resolution of all target PAH analytes and significantly improved peak height response compared to a comparable column packed with full-porous media.

Questions? Make sure to reach out to our Technical Experts for nearly 24/7 help!

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References

> Bringing the Performance Advantage of Core-Shell Technology to PAH Analysis-Part 1

> Performance Advantage of Core-shell Technology to PAH Analysis-Part 2

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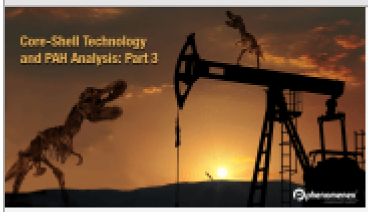
Summary




Article Name

Performance Advantage of Core-Shell To PAH Method Analysis-Part 3

Description

In this final part of Dr. Jeff Layne's series on core-shell technology used to advance performance of PAH method analysis, we focus on EU 15+1 PAH Method.