Polycyclic Aromatic Hydrocarbon (PAH) Separations Using ZORBAX Eclipse PAH Columns – Analyses from Six to 24 PAHs

Application

Food Safety

Authors

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Abstract

Different mixtures of polycyclic aromatic hydrocarbons (PAHs) were separated by reversed phase HPLC, using ZORBAX Eclipse PAH columns and an acetonitrile / water mobile phase. These analyses include combinations of six PAHs up to 24 PAHs. To separate this range of PAHs, both gradient and temperature adjustments were used to manipulate resolution. For these separations, optimum column choices – in a variety of column dimensions – are available to food and environmental chemists, resulting in improved and optimized resolution, analysis time, detector choice, sample size, or system pressure.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed by the incomplete burning of hydrocarbons such as coal, gas, oil, and wood. They are generated from automobile exhaust, forest fires, and even grilled meats (charring and smoke). They are usually found as a mixture, and broadcast in the air as particles and soot.

Given that PAHs have limitless origins and are likely carcinogenic, many government agencies such as Occupational Safety and Health Administration (OSHA), National Institute of Occupational Safety and Health (NIOSH), and European Union's Scientific Committee on Food have proposed or mandated exposure limits; thus, analytical methods including HPLC are being improved to monitor PAHs.

The broad range of PAH sample matrices (air, water, soil, and food) and the large number of PAHs (over 100 compounds) requires different analytical techniques. Fortunately, many PAH HPLC methods can be developed with Eclipse PAH columns because of the numerous column dimensions and excellent scalability, reproducibility, and longevity. HPLC methods are useful for PAH analysis because UV and fluorescence detection offers enhanced selectivity by UV and fluorescence spectra over other techniques such as GC with flame ionization detection.

The most dominant example of PAHs has been the 16 separated in the EPA 610 method. But for food and environmental analysis, both subsets of this set and additional PAHS may need to be separated. The five examples of different mixtures of PAHs shown here include a food screening method of only six PAHs and a more complete analysis method that can be used for food that includes up to 18 PAH compounds. In addition, three different environmental separations are shown starting with the 16 PAHs and including samples with up to 24 PAHs.

All of these samples were separated with ZORBAX Eclipse PAH columns by changing the mobile phase gradient and the column dimensions for optimum resolution and analysis time. An acetonitrile/water mobile phase is used for all analyses as this is the typical mobile phase for many standardized PAH methods such as EPA 610 and EPA 8330. Varying column temperature was demonstrated to change selectivity and is useful for optimizing PAH methods, particularly the more complex method for potential PAHs in food. Several column configurations are used to demonstrate method customization, emphasizing speed, resolution, or column choices for HPLC instruments with different pressure limits. Sample matrix and sample concentration are other variables that influence choice of column dimensions.

Experimental

PAH separations were developed on ZORBAX Eclipse PAH columns and an Agilent Rapid Resolution 1200 Series LC (RRLC) system comprising a:

- G1379 degasser
- G1312B binary pump SL
  - Mobile phase channel A: water, B: acetonitrile. See figures for gradient conditions.
  - When using 2.1-mm id columns, the pump was configured in the low-delay volume mode, bypassing the static mixer and pulse dampener. See reference 1 for details about using low- and standard-volume binary pump configurations.
- G1367C HiP-ALS SL Autosampler
- G1316B TCC SL Thermal Controlled Column Compartment
  - Temperature: 25 °C
  - When using 2.1-mm id columns, the low-volume (1.6 µL) heat exchanger (G1316-8002) was used in place of the built-in 3-µL one.
- G1315C diode array detector SL
  - Set at “220 nm, 4 nm, no reference,” with a G1315-60025 flow cell (5-µL volume), response time setting of 0.5 s
- G1321A FLD fluorescence detector
- See figures for columns used and other specific method parameters.

The PAH mixtures were purchased as ready made solutions or were made by combining individual standards. Dissolution of neat compounds was in toluene or methylene chloride. Dilutions were done with ethanol. The variety of PAHs were obtained from several commericial sources:

- Figure1: PAH Mixture (Agilent PN 8500-6035)
- Figure 2: PAH Mixture (Agilent PN 8500-6035) with terphenyl-d14 (Ultra Scientific PN ATS-160-1), 1-methylnaphthalene (Ultra Scientific PN EPA-1225), 2-methylnaphthalene (Ultra Scientific PN SV-200-1) and benz(e)pyrene (AccuStandard H-112S)
- Figures 3 and 4: Quebec Ministry of Environment PAH Mix (AccuStandard PN H-QME-01)
- Figures 5 to 7: Made from solutions or neat PAH compounds from AccuStandard:

Benzo[c]phenathrene	H-244N
Triphenylene	H-235N
Benz[a]anthracene	Z-013-04
Chrysene	H-115N
Benzo[b]flouranthrene	H-128N
Benzo[k]flouranthrene	H-129N
Benzo[a]pyrene	H-169N
Benzo[g,h,i]perylene	H-103N
Indeno[1,2,3-cd]pyrene	H-157N
Dibenzo[a,i]pyrene	H-178N
Dibenzo[a,h]pyrene	H-177N
From Cerilliant:	
Cyclopenta[c,d]pyrene	SCC-048
5-methylchrysene	ERM-028
Benzo[j]flouranthrene	ERB-005
Dibenzo[a,l]pyrene	ERD-051
Dibenzo[a,h] anthracene	ERD-052
Dibenzo[a,e]pyrene	ERD-009
From Sigma-Aldrich:	
Benzo[c]fluorene	T175560

Results and Discussion

The ruggedness of the ZORBAX Eclipse PAH columns was previously shown for actual separations of PAHS. Longevity, batch-to-batch reproducibility, and constant selectivity between three particle sizes was demonstrated using the EPA priority pollutants PAH standard mix (EPA 610) [2]. Figure 1 shows the separation of the PAHs at three different sample loads using the fluorescence

detector (FLD). Sensitivity (signal-to-noise ratio > 10:1) of that method using the FLD detector is in the picogram range (Figure 1). Limit of detection by UV is about 100-fold less [3]. Only 15 of the 16 PAHs are detected because acenaphthylene (peak 2) does not fluoresce. Specific settings for optimum results with the FLD are shown on the chromatogram and include photomultiplier tube (PMT) settings. The information is listed for the chromatogram with 10 ng on column, and settings were adjusted as labeled in the chromatograms with 500 and 50 picogram amounts on column.

Four additional PAHs are combined with the EPA 610 mix to make a 20-compound standard for an application-specific method. Two of the PAHs, (1-methylnaphthalene and 2-methylnaphthalene) when added to the EPA 610 mix, are a PAH mixture described in the Florida Administrative Code, chapter 17.700, which deals with remediating soil. The other two PAHs added were terphenyl and benzo[e]pyrene. Figure 2 shows the chromatogram on the Eclipse PAH column, resolving all 20 analytes. The gradient was adjusted in comparison to one typically used for 16 PAHs in order to achieve complete resolution of the additional analytes.

A more complex PAH mixture, the Quebec Ministry of the Environment PAH standard, contains 24 PAHs. It was separated and the results are shown in Figure 3. This separation was produced using a Rapid Resolution Eclipse PAH 4.6 mm × 150 mm, 3.5-µm column. The advantage of this Rapid Resolution (RR, 3.5 µm) configuration is lower pressure compared to 1.8-µm columns, so that the long RR column can run on HPLC systems such as the Agilent 1100 and Agilent 1200 (non-RRLC), because the operating pressure is under 400 bar.

Similar resolution of the critical pairs, (peaks 13, 14, and 15) and significant time savings can be achieved if the shorter Rapid Resolution High Throughput (RRHT, 1.8 µm) 4.6 mm × 100 mm column is used (Figure 4). The column id was kept the same so the gradient method was shortened proportional to the column length. An Agilent 1200 RRLC system is needed because the pressure is over 400 bar.

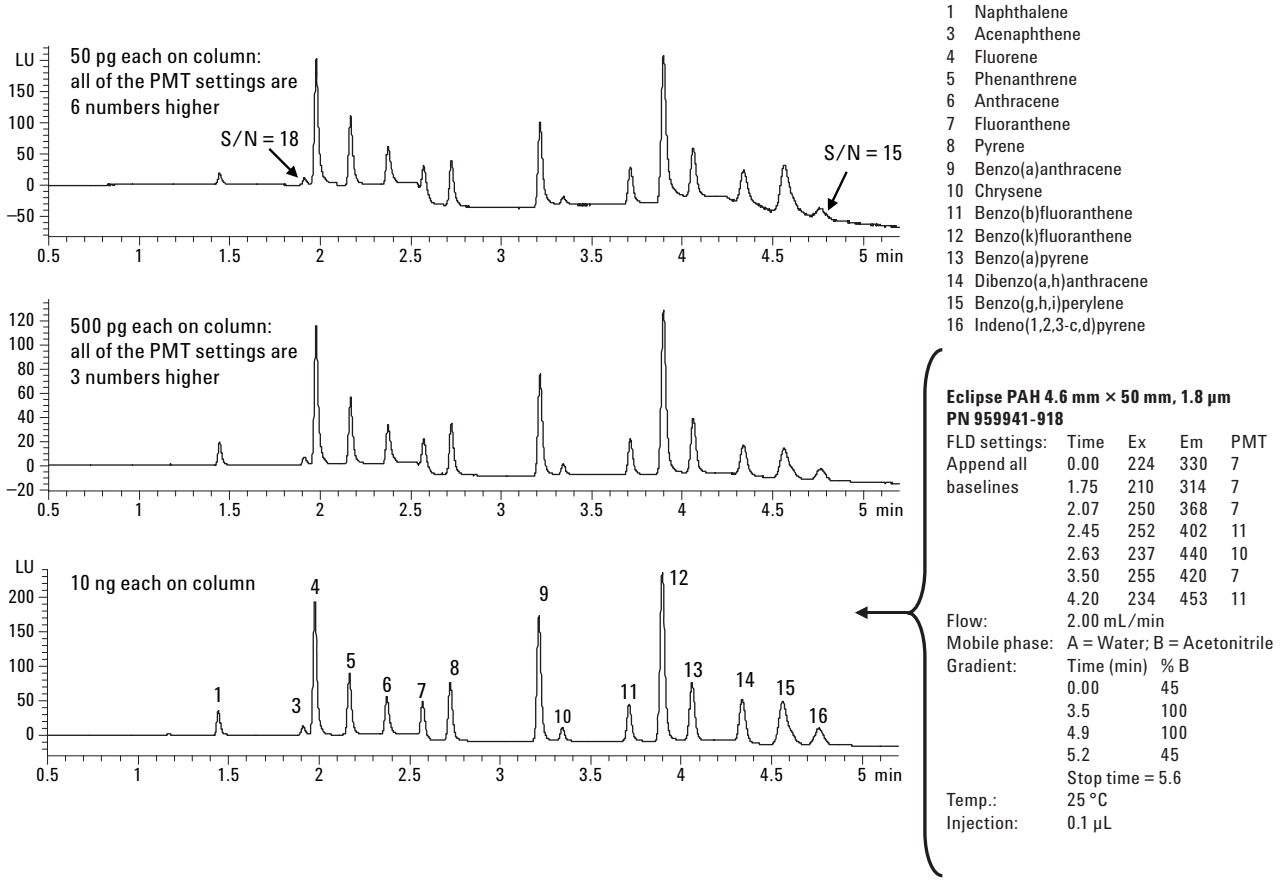


Figure 1. Quantitative sensitivity of FLD detection: S / N ≥ 10 of EPA method 610 PAHs on an Eclipse PAH 4.6 mm × 50 mm, 1.8 µm column.

Another complex analysis of 18 PAHs is shown in Figure 5. This is a combination of the EU "15+1" PAH (the 15 PAHs recommended for monitoring by the European Union's (EU) Scientific Committee on Food (SCF), plus benzo[c]florene, added in 2005 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [4]), and two additional PAHs (triphenylene and benzo[c]phenanthrene). All 18 PAHs can be resolved on an Eclipse PAH column (2.1 mm × 50 mm, 1.8 µm) with a simple gradient in less than 9 minutes. The column selected here was also a RRHT, 1.8-µm column, but the pressure did not exceed 400 bar because of the short column length and the mobile phase used.

Temperature is a useful tool to change selectivity. Note that the resolution factor of the critical pair (peaks 2 and 3) in Figure 5 is 1.44 at 25 °C. It has often been noted that with the polymeric bonded phases used for PAH columns that lowering the temperature improves resolution; that occurred here for increased resolution of peaks 2 and 3. Figure 6 overlays the separation at three temperatures: 25, 20, and 15 °C. The resolution factor of the critical pair improves from 1.44 to 2.01 and 2.52, respectively. Peaks 13 and 14 elute closer together, however, as temperature is lowered. A good compromise that offers satisfactory resolution for both pairs is to operate at 20 °C. Noticeable chromatographic differences with just a few degrees change in temperature indicate that temperature should be carefully controlled, especially when operating at ambient temperature, as room temperature may fluctuate in laboratories. The maximum operating temperature for the Eclipse PAH is 60 °C (below pH 6), but most PAH separations will be optimized between 15 and 35 °C with this column.

Our last example is related to the European Community Directive 80/778/EEC, regarding the quality of drinking water, and calls for rapid screening of water samples for PAHs by isocratic analysis. High throughput is accomplished using short columns and an isocratic mobile phase to eliminate gradient re-equilibration time. An RR column configuration (3.5 µm) separates the sample in about four minutes, and an RRHT column (1.8 µm) reduces analysis time to two minutes, with no loss of resolution (Figure 7). Because the columns are short and the acetonitrile concentration is high, high pressure is not an issue with either column, even at the fast flow rates.

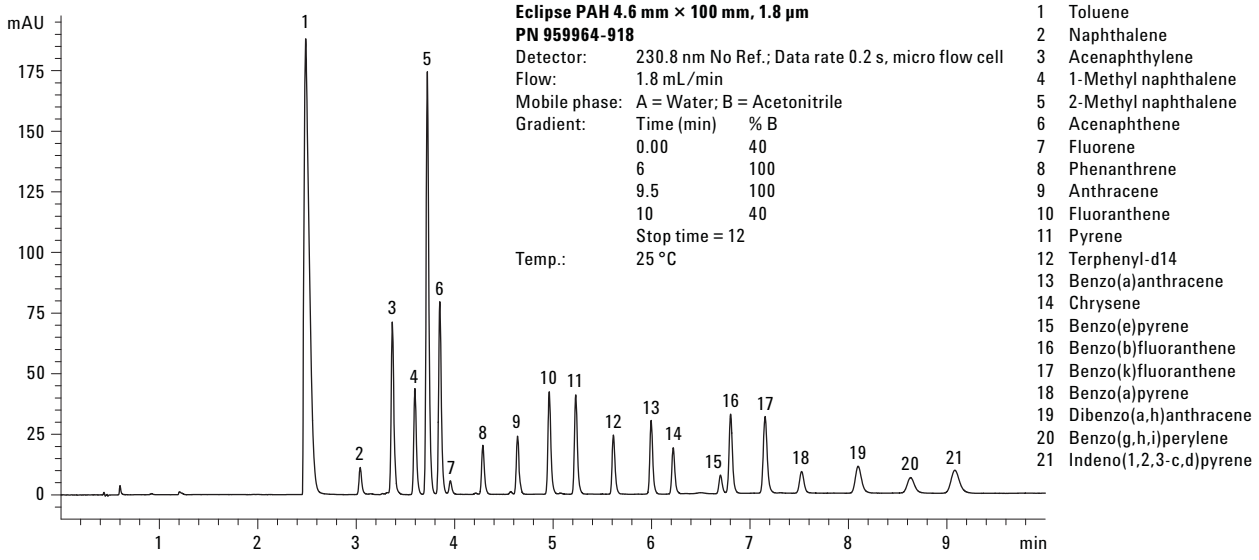


Figure 2. RRHT analysis of Florida Administrative Code 17.000, including two additional PAHs.

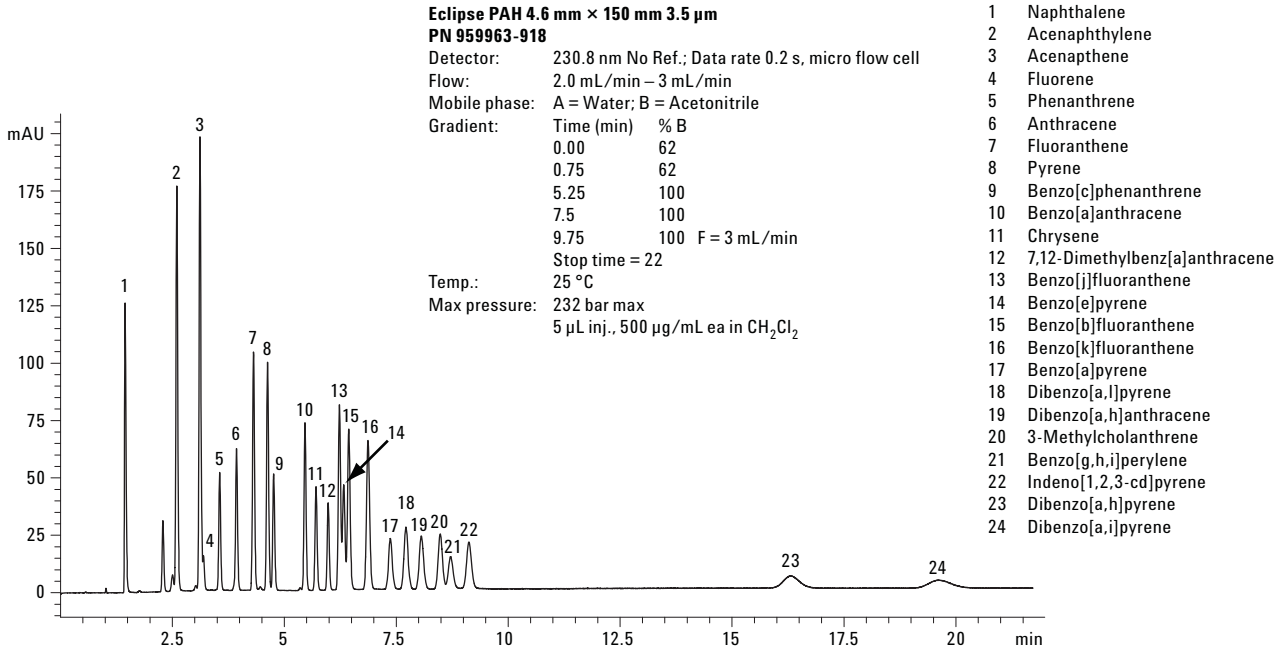


Figure 3. Quebec Ministry of Environment PAH standard RR analysis option.

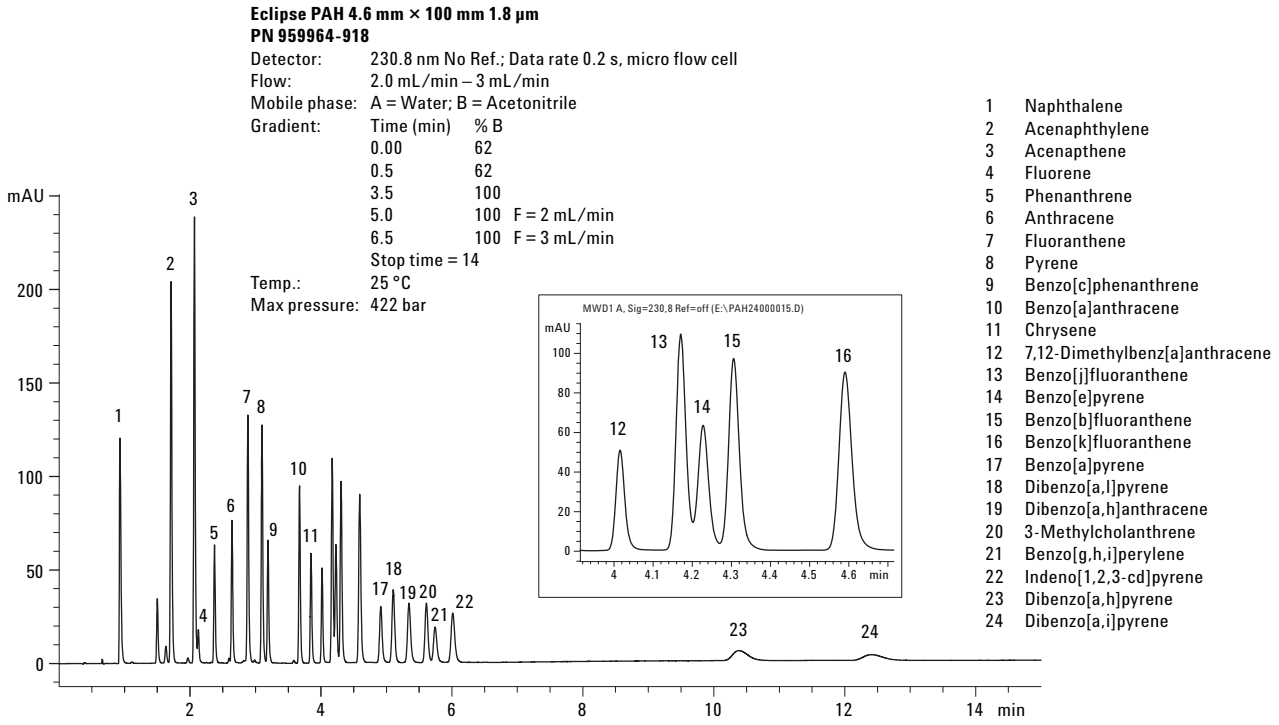


Figure 4 Quebec Ministry of Environment PAH standard RRHT analysis option.

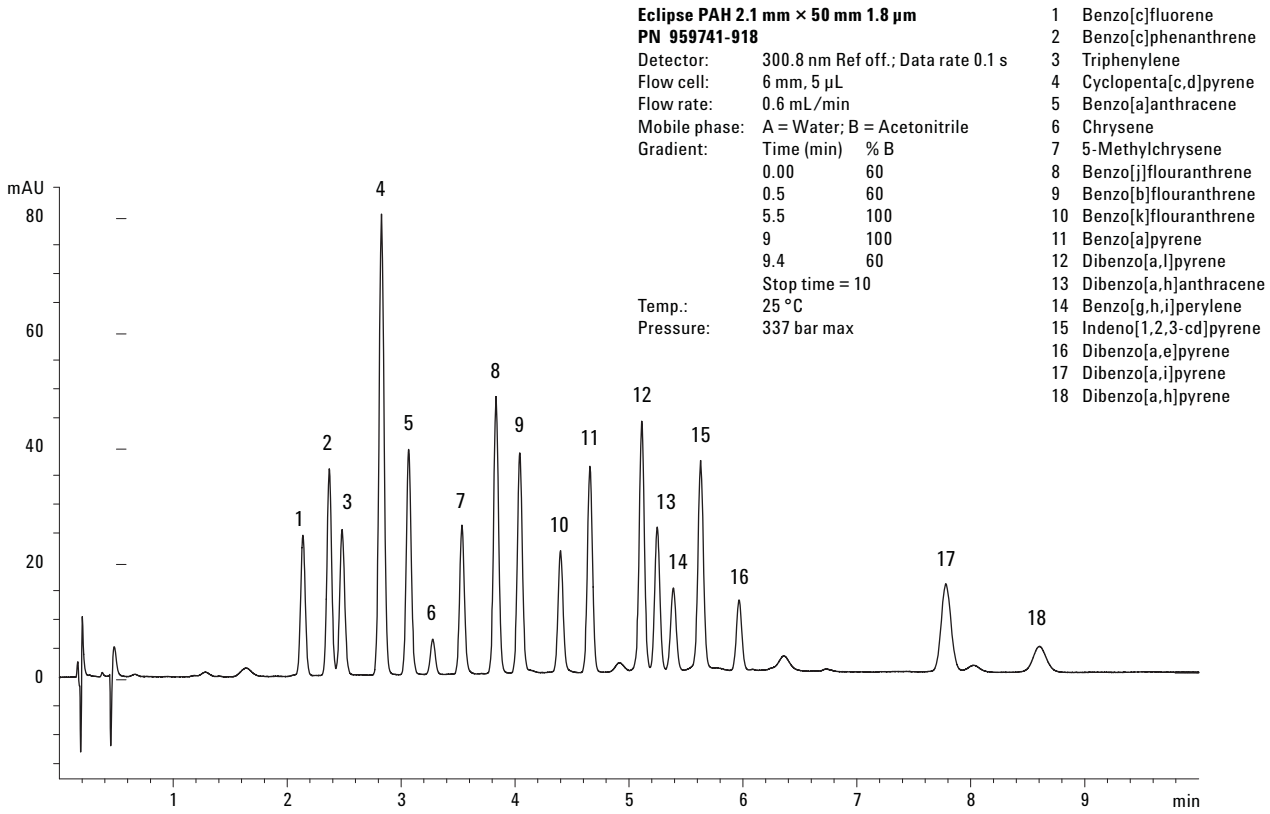


Figure 5. The EU's SCF and JECFA "15+1" with two additional PAHs.

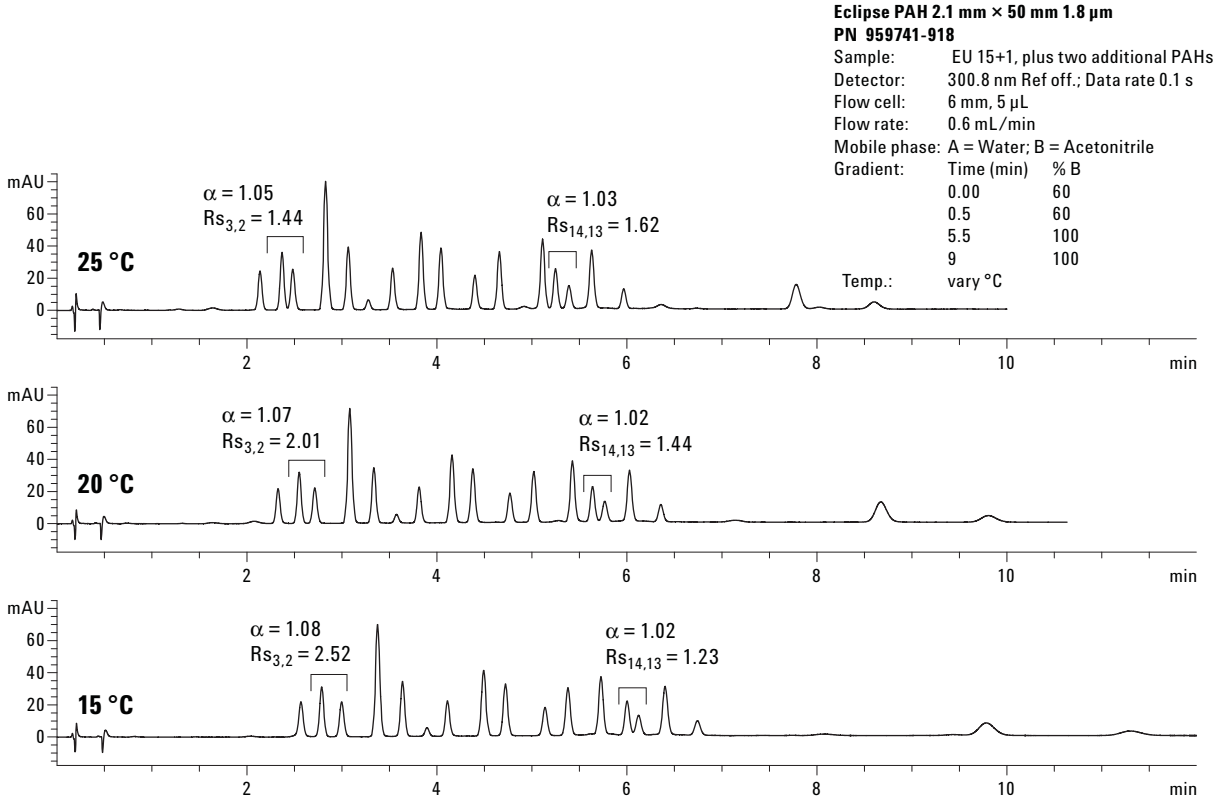
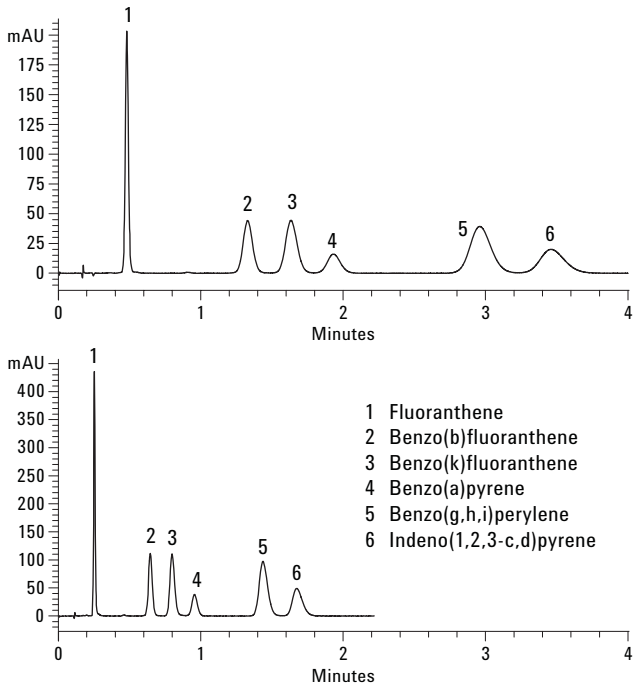


Figure 6. Effect of temperature on band spacing of PAHs.



Eclipse PAH, 4.6 mm × 50 mm, 3.5 µm
PN 959943-918
Detector: 220.4 nm
Flow: 2.5 mL/min
Channel: A = Water; B = Acetonitrile
Mobile phase: 92% B
Temp.: 20 °C, low vol heat sink
Pressure: 105 bar

Eclipse PAH, 4.6 mm × 30 mm, 1.8 µm
PN 959931-918
Detector: 220.4 nm
Flow: 2.5 mL/min
Channel: A = Water; B = Acetonitrile
Mobile phase: 95% B
Temp.: 20 °C, low vol heat sink
Pressure: 147 bar

Figure 7: Rapid resolution PAH screening columns for European Community Directive 80/778/EEC.

Conclusions

ZORBAX Eclipse PAH columns' proven robustness makes them ideal for the broad variety of PAH samples in matrices such as air, water, soil, and food.

Five examples of PAH mixtures from environmental and food safety industries and ranging in complexity from six to 24 PAHs were successfully separated on different sizes of Eclipse PAH columns. Gradient composition, temperature, and column dimensions were easily manipulated for method development and optimization. The breadth of Eclipse PAH column configurations is useful to customize speed and resolution or complement the sample matrix, sample concentration, or detector choice.

- Eclipse PAH columns available for optimization:
  - 959764-918 Eclipse PAH, 2.1 mm × 100 mm, 1.8 µm
  - 959793-918 Eclipse PAH, 2.1 mm × 100 mm, 3.5 µm
  - 959763-918 Eclipse PAH, 2.1 mm × 150 mm, 3.5 µm
  - 959701-918 Eclipse PAH, 2.1 mm × 150 mm, 5 µm
  - 959790-918 Eclipse PAH, 2.1 mm × 250 mm, 5 µm
  - 959741-918 Eclipse PAH, 2.1 mm × 50 mm, 1.8 µm
  - 959990-318 Eclipse PAH, 3.0 mm × 250 mm, 5 µm
  - 959964-918 Eclipse PAH, 4.6 mm × 100 mm, 1.8 µm
  - 959961-918 Eclipse PAH, 4.6 mm × 100 mm, 3.5 µm
  - 959996-918 Eclipse PAH, 4.6 mm × 100 mm, 5 µm
  - 959963-918 Eclipse PAH, 4.6 mm × 150 mm, 3.5 µm
  - 959993-918 Eclipse PAH, 4.6 mm × 150 mm, 5 µm
  - 959990-918 Eclipse PAH, 4.6 mm × 250 mm, 5 µm
  - 959931-918 Eclipse PAH, 4.6 mm × 30 mm, 1.8 µm
  - 959941-918 Eclipse PAH, 4.6 mm × 50 mm, 1.8 µm
  - 959943-918 Eclipse PAH, 4.6 mm × 50 mm, 3.5 µm

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References

1. "High-Throughput Gradient Optimization by Easily Minimizing Delay Volume," Agilent Technologies publication 5989-6665EN (2007)
2. "Robustness of Eclipse PAH Columns," Agilent Technologies publication 5989-7828EN (2008)
3. "Polynuclear Aromatic Hydrocarbons (PAHs) Analysis in Water with ZORBAX Eclipse PAH HPLC Column," Agilent Technologies publication 5989-7953EN (2008)
4. "Analytical methods for polycyclic aromatic hydrocarbons in food and the environment needed for new food legislation in the European Union," *Trends in Analytical Chemistry*, Vol. 25, No. 7, 2006

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