Robustness of Eclipse PAH Columns for HPLC Analysis of Polycyclic Aromatic Hydrocarbons

Application

Environmental and Food

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Abstract

The Eclipse PAH (polycyclic aromatic hydrocarbon) column contains a rugged stationary phase suitable for a variety of PAH analyses. Their longevity, reproducibility, and scalability were demonstrated. The large number of available column configurations makes Eclipse PAH columns a desirable first choice to satisfy the chromatographer's unique PAH analytical requirements.

Introduction

Some HPLC column manufacturers offer a specific column for polycyclic aromatic hydrocarbon (PAH) analysis. However, because of the large number of PAHs (over 100 compounds) and broad range of PAH matrices (such as air, water, and food), many unique HPLC methods are needed, and cannot be developed on just one or two PAH column configurations. The more column lengths and diameters available, the more rugged, optimized methods can be developed.

More importantly, particle size, an additional column dimension, greatly expands method customization, meaning even more methods can be generated and optimized for a particular PAH application. Uniform chemistry between particle sizes allows methods to be scaled up or down with predictable results. Additional column possibilities are useful for such factors as sample size, sample matrix, detector choice, speed, resolution, and solvent use.

For a specialty column (bonded phase) to be useful for a multitude of similar methods it must demonstrate reproducibility between particle sizes. This allows straightforward, predictable method transfer, minimizing method redevelopment. Ideally, the column should also have longevity and reproducibility, including between manufacturing lots.

In this work we demonstrate that Eclipse PAH columns are robust: they have long life, reproducibility, and scalability.

Experimental

Eclipse PAH ruggedness was tested on an Agilent Rapid Resolution 1200 Series LC (RRLC) system that comprised:

- G1379 degasser

- G1312B binary pump SL
  - Mobile phase A: water, B: acetonitrile. See figures for gradient conditions.
  - When using 2.1-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
  - Set to 25 °C. When using 2.1-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, 4 nm, no reference, with a G1315-60025 flow cell (5-µL volume), response time setting of 0.5 s

Eclipse PAH Columns Available

Part Number	Description
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

See figures for columns used.

The PAH mixture is a certified reference material from Agilent, PN 8500-6035, diluted in acetonitrile. Elution order for all figures:

1 Toluene
2 Naphthalene
3 Acenaphthylene
4 Acenaphthene
5 Fluorene
6 Phenanthrene
7 Anthracene
8 Fluoranthene
9 Pyrene
10 Benzo(a)anthracene
11 Chrysene
12 Benzo(b)fluoranthene
13 Benzo(k)fluoranthene
14 Benzo(a)pyrene
15 Dibenzo(a,h)anthracene
16 Benzo(g,h,i)perylene
17 Indeno(1,2,3-c,d)pyrene

Results and Discussion

The standard mixture of polynuclear hydrocarbons specified in the EPA method 610 for municipal and industrial wastewater evaluated the robustness of Eclipse PAH columns. EPA method 610 calls for a 2.6 mm × 250 mm, 5 µm ODS column and a water/acetonitrile gradient. Alternative columns are allowed if certain conditions are met [2]. Interestingly, reference 2, section 1.3, states that the LC method with its specified column does not resolve all 16 PAHs.

Eclipse PAH does resolve all 16 PAHs, even in a 5-µm, 250-mm long configuration (Figure 1). Note that the critical pair, peaks 4 and 5, is well resolved ($R_s > 2$). We chose this minimum resolution of the critical pair to define a successful robust method. Mobile phase was adjusted to obtain this resolution for all Eclipse PAH column configurations; thus, the wide range of gradient delay times between low and high flow rates would not be a concern when developing the separation on a different column dimension. The analysis in Figure 1 takes about 26 minutes on the long 250-mm column. The analysis was shortened over four-fold when 1.8-µm particles in a 50-mm long column were used (Figure 2). The resolution of the critical pair remains greater than 2, but analysis time was reduced to 6.8 min.

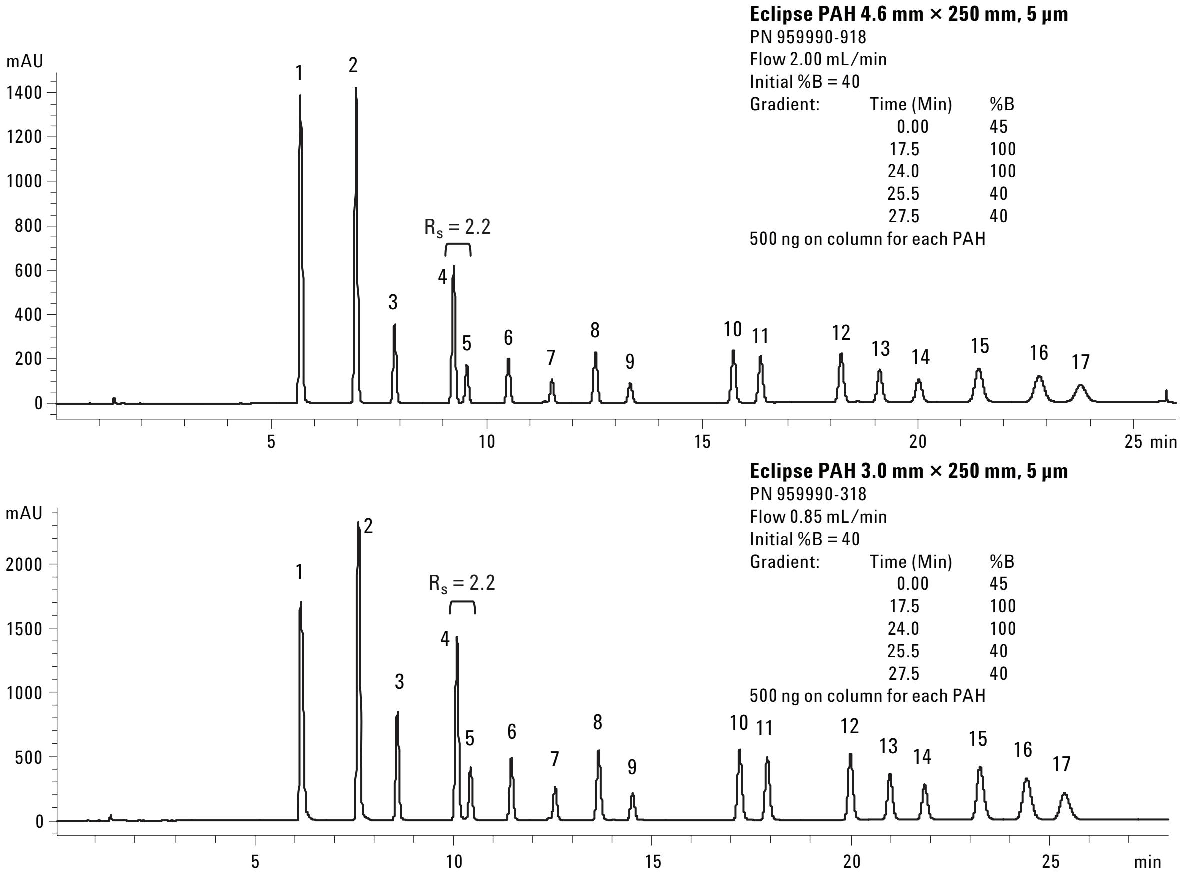

Figure 1. PAH analysis on Eclipse PAH 250-mm columns has high resolution.

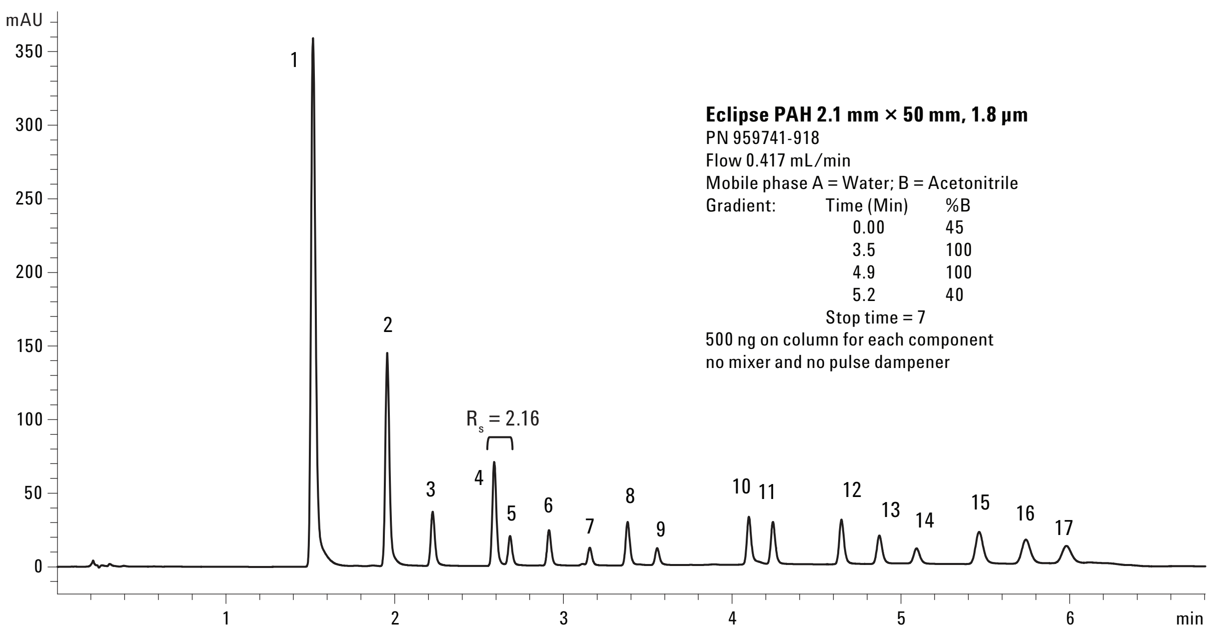

Figure 2. PAH analysis on RRHT Eclipse PAH 2.1 mm × 50 mm 1.8-µm column also has high resolution and faster analysis time.

Long Life and Reproducibility

Low solvent consumption and high throughput gained by using Rapid Resolution High Throughput (RRHT) columns such as the Eclipse PAH 2.1 x 50 mm in Figure 2 make RRHT columns ideal for column lifetime tests. We used the method in Figure 2 to test the longevity of Eclipse PAH columns. After 5,000 injections, the test was terminated with little loss in column performance. Figure 3 overlays chromatograms from the beginning, middle, and end of the life test. Selectivity, retention, and efficiency, and therefore resolution, remained relatively constant for 3,000 analyses and remained quite satisfactory for the next 2,000 injections. The table in Figure 3 lists the resolution factor values of the critical pair and a wider spaced pair and supports the method and column robustness. The test took 25 days of 24-hour operation and generated roughly 14.6 L of solvent waste. If a 4.6 x 250 mm column had been used, the test would have taken 122 days of nonstop operation and about 350 L of solvent would have been consumed.

Batch-to-Batch Reproducibility

Long column life is an important feature of Eclipse PAH; another necessity is batch-to-batch reproducibility. Besides 5- and 1.8-µm particles, Eclipse PAH is also available in 3.5 µm. Figure 4 compares two batches of 3.5-µm Eclipse PAH material made at different times. Note that the selectivity is identical between the batches, supporting the claim that manufacture of the Eclipse PAH particles is uniform. Similar results were obtained from 5- and 1.8-µm material (data not shown). Each batch of material is specifically tested with PAHs for maximum reproducibility under expected operating conditions.

Scalability Between Particle Sizes

Batch-to-batch reproducibility can be broadened to particle-size-to-particle-size reproducibility, or scalability, to fully appreciate a column's robustness. Figure 5 overlays three different particle-size columns (by definition, three distinct batches as well). Additionally, the columns comprise three lengths and two diameters. Selectivity is the same, however, for all three column configurations. This is because selectivity is related to the nature of the particle surface, not to column length or diameter. The uniform selectivity between particle sizes, or scalability, contributes to Eclipse PAH's robustness.

Run #	$Rs_{5,4}$	$Rs_{11,10}$
5000	1.75	2.52
4000	1.81	2.61
3000	1.97	2.85
2000	1.95	2.75
1000	2.01	2.85
1	2.09	2.85

Agilent 1200SL DAD 220,4 nm No Ref. DAD		
Stop time	7.0 min	
Flow	0.417 mL/min	
Mobile phase A	Water	
Mobile phase B	Acetonitrile	
Gradient	Time (min)	%B
	0.00	45
	3.5	100
	4.9	100
	5.2	45
Stop time	7	
Temperature	25 °C	
No mixer or pulse dampener		

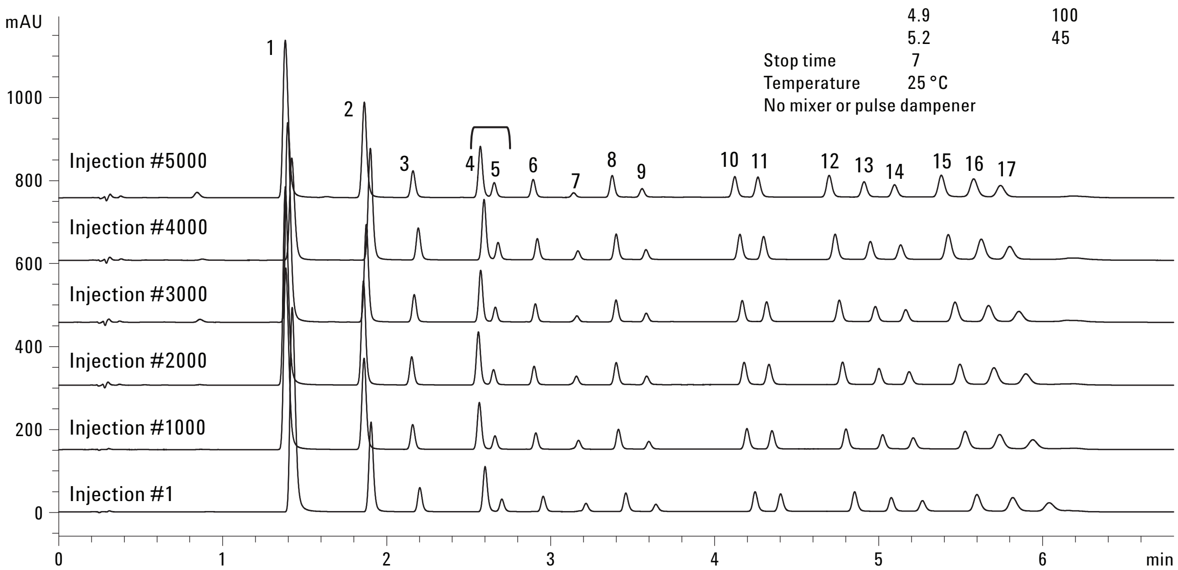

Figure 3. Life test of Eclipse PAH 2.1 x 5 mm, 1.8 µm. See the Experimental Section for peak identification.

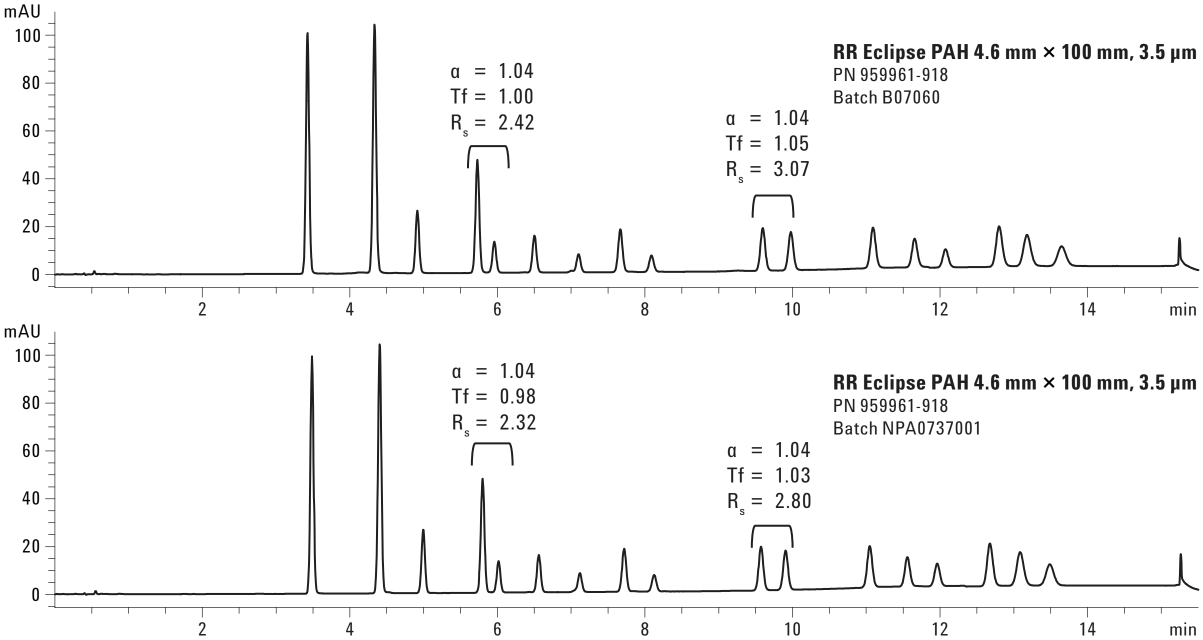

Figure 4. Batch-to-batch reproducibility of Eclipse PAH 3.5-µm material.

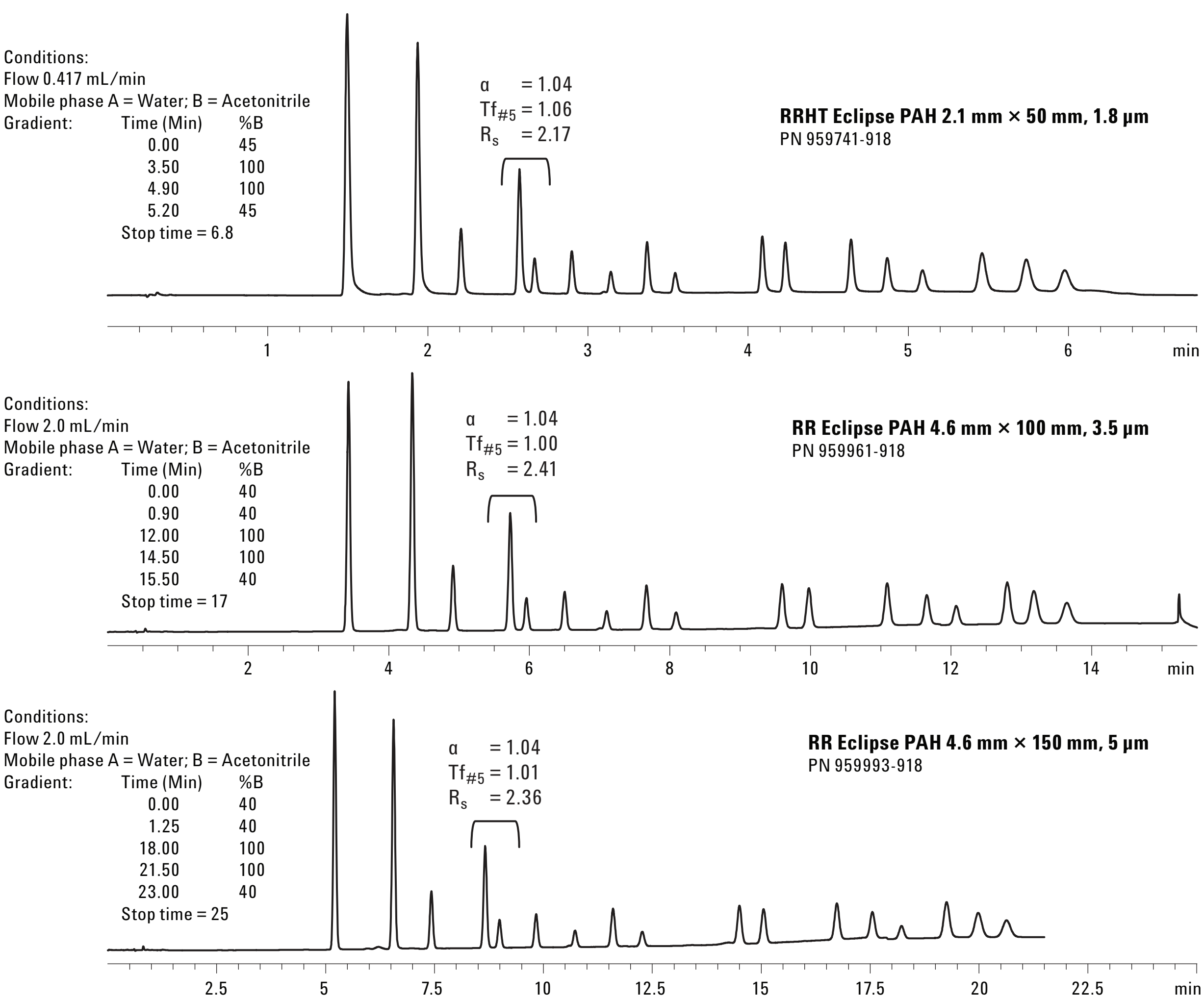

Figure 5. Scalability of Eclipse PAH columns of various particle sizes and column dimensions.

Conclusions

Eclipse PAH is a suitable rugged stationary-phase column for a wide variety of PAH analyses. Column longevity, reproducibility, and scalability were demonstrated. The large number of column configurations makes Eclipse PAH columns a first choice for method optimization. Available Eclipse PAH column dimensions allow method customization regarding sample size, matrix, detector type, analysis speed, resolution requirements, and solvent consumption.

References

1. "High-Throughput Gradient Optimization by Easily Minimizing Delay Volume," Agilent Publication 5989-6665EN (2007)
2. Appendix A to Part 136, Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, Method 610 Polynuclear Aromatic Hydrocarbons, United States Environmental Protection Agency

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