Rapid Method Development for 18 PAH Compounds with an Agilent RRHD Eclipse PAH Column

Application Note

Environmental

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Abstract
A fast screening of gradient times with 18 PAH compounds is possible within the 1,200 bar pressure limits of both an Agilent ZORBAX RRHD Eclipse PAH column and an Agilent 1290 Infinity UHPLC. An acetonitrile/water gradient is used; as this is the typical mobile phase used for many standard PAH analyses, such as EPA 610 and EPA 8330. Resolution and conditional peak capacity are calculated and used to evaluate the various gradients.
Introduction

Polycyclic aromatic hydrocarbons (PAHs) are known to possess mutagenic and carcinogenic properties. PAHs are generated by the incomplete burning of hydrocarbons, such as coal, gas, oil and wood. These compounds are then introduced to the environment, where they can be found in soil, water and air. Determination of these hazardous PAHs in each of these matrices is an important role for environmental laboratories.

Advancements in liquid chromatography have led to significantly improved sample throughput, which is advantageous to many environmental laboratories. Agilent Technologies’ 1290 Infinity UHPLC and Agilent ZORBAX Rapid Resolution High Definition (RRHD) columns are manufactured to withstand pressures up to 1,200 bar.

Recently, the Agilent ZORBAX RRHD Eclipse PAH column has become available. This PAH column uses the same chemistry as previous Eclipse PAH columns; however, it has the added benefit of 1,200 bar stability. These columns are good for applications requiring the separation of geometric isomers, and are specifically tested with PAHs for maximum reproducibility under expected operating conditions. Additionally, the numerous column dimensions and particle sizes available for Eclipse PAH allow for maximum scalability of any optimized PAH analysis.

Experimental

An Agilent 1290 Infinity UHPLC with an Agilent ZORBAX RRHD Eclipse PAH 2.1 x 100 mm, 1.8 µm column (p/n 959758-918) was used in this experiment.

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>A: water</th>
<th>B: acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>0.84 mL/min</td>
<td></td>
</tr>
<tr>
<td>Gradient</td>
<td>40–100% B, gradient time (t_g) varies from 1 to 20 minutes; isocratic hold at 100% B for 2 minutes, re-equilibrate column at 40% B for 3 min</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>0.5 µL injection of diluted Agilent PAH Mixture (p/n 8500-6035) spiked with thiourea as a v_0 marker</td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>25 °C</td>
<td></td>
</tr>
<tr>
<td>DAD</td>
<td>Sig = 220.4 nm; Ref = Off</td>
<td></td>
</tr>
</tbody>
</table>

MassHunter versions B.03.01, B.02.00 and B.03.01 were used for data acquisition, qualitative and quantitative analyses, respectively.

Several gradient methods were compared for the separation of 18 PAH compounds. The quality of each gradient were evaluated using two parameters: resolution (Equation 1) and conditional peak capacity (Equation 2).

Resolution is the measure of the separation between two peaks. Ideally baseline separation is Rs = 2. Minimum resolution will be used to evaluate each analysis.

\[
Rs = 2\left(\frac{t_{Rj} - t_{Ri}}{W_i + W_j}\right)
\]

where: \(t_{Ri}\) and \(t_{Rj}\) = retention time of peaks i and j respectively
\(W_i\) and \(W_j\) = peak width at baseline of peaks i and j respectively

Peak capacity will also be used to evaluate each analysis, and is defined as the number of peaks that can be theoretically separated within a gradient time.

\[
N_C = \frac{t_{Rn} - t_{R1}}{W}
\]

where: \(t_{Rn}\) = retention time of last eluting peak
\(t_{R1}\) = retention time of first eluting peak
\(W\) = average 4σ peak width = 4(W_1/2 / 2.35)

Results and Discussion

Method development for 18 PAH compounds is shown in Figure 1. A typical acetonitrile/water gradient for PAH compounds (40–100% CH_3CN) is screened to quickly determine an acceptable analysis method. The analyses are evaluated with regards to conditional peak capacity and minimum resolution. As can be seen, longer gradient times yield higher conditional peak capacity \(N_C\) measurements. Peak capacity is an indication of how many peaks can hypothetically be separated over a given gradient time. While conditional peak capacity is a good measure of quality for a gradient analysis, it should not be the only qualification, as resolution is equally, if not more important for a UV method like this PAH analysis. Baseline separation of two compounds is generally indicated by a resolution of at least two. Only the 5 and 10 min gradients provide a minimum resolution greater than two, with the 5 min gradient producing the best minimum resolution of 2.12. While different laboratories will require different qualifications for any given analysis, in this example the 5 min gradient PAH analysis appears to achieve a nice balance of desirable chromatographic characteristics for 18 PAH compounds in a total cycle time of 10 min (gradient + isocratic hold + re-equilibration).
**Figure 1.** Gradient times are rapidly screened for the separation of 18 compounds on an Agilent RRHD Eclipse PAH 2.1 x 100 mm, 1.8 µm column, see Experimental section for detailed method parameters.

### Conclusion

Gradient times can quickly be screened with this Agilent ZORBAX RRHD Eclipse PAH column and an Agilent 1290 Infinity UHPLC, each with a 1,200 bar pressure limit. In order to achieve baseline separation of all 18 PAH compounds (minimum resolution of 2), either a 5 or 10 minute gradient must be considered. A gradient scan like this, performed on a sub-2-µm column within a 600 bar pressure limit would take at least twice the amount of time as this screening, which was performed around a maximum pressure of 1,020 bar. Larger particles or shorter columns could be considered within a 600 bar range, however resolution will likely be lost if run at these same fast flow rates and gradient times.

### For More Information

These data represent typical results. For more information on our products and services, visit our Web site at [www.agilent.com/chem](http://www.agilent.com/chem).