GC/MS Analysis of European Union (EU) Priority Polycyclic Aromatic Hydrocarbons (PAHs) using an Agilent J&W DB-EUPAH GC Column with a Column Performance Comparison

Application Note

Environmental

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Abstract

Recent European legislation has established a list of polycyclic aromatic hydrocarbons (PAHs) to be monitored in foods. These target PAHs can prove chromatographically problematic with non-polar GC columns typically used for the analysis of US EPA priority PAHs. This application demonstrates a GC/MS method for the determination of the 15+1 European Union (EU) priority PAHs using the application-specific Agilent J&W DB-EUPAH GC column. This column provided baseline resolution for critical pairs and excellent sensitivity for the heavier PAHs. In addition, DB-EUPAH showed improved bleed performance and better signal-to-noise ratios when compared to a Restek Rxi-17 column. This resulted in more accurate quantitation especially for late eluting dibenzopyrenes.

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Introduction

Polycyclic aromatic hydrocarbons are a large class of organic compounds containing two or more fused aromatic rings. PAHs are often formed from the incomplete burning of organic substances such as wood, coal, and oil. A main source of human exposure to PAHs is food, through the heat processing of meat and dairy products, such as grilling and smoking [1]. Serious health concerns have arisen as many PAHs have been classified as carcinogenic or mutagenic [2].

Most current analytical methods focus on the sixteen PAHs the US Environmental Protecting Agency (EPA) targeted in the 1970s [3]. In 2005, the European Commission recommended the monitoring of fifteen EU priority PAHs along with an additional PAH highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [4]. These 15+1 EU priority PAHs include benzo[c]fluorene, benz[a]anthracene, cyclopenta[c,d]pyrene, chrysene, 5-methylchrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benz[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene, and dibenzo[a,h]pyrene. Eight of these compounds (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benz[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene) are also listed in the 16 USEPA-regulated PAH list.

A 5% phenyl methylpolysiloxane stationary phase is the most commonly used GC column for PAH analysis. This non-polar column yields good resolution for the 16 USEPA PAHs [5,6], however, three critical pairs of the 15+1 EU PAHs co-elute and are difficult to resolve [7,8]. These challenging pairs are benz[a]anthracene-cyclopenta[c,d]pyrene- chrysene, benzo[b]fluoranthene-benzo[k]fluoranthene-benzo[j]fluoranthene, and indeno[1,2,3-cd]pyrene-dibenz[a,h]anthracene. Agilent J&W DB-EUPAH, a midpolar GC column, improves the resolution of these critical pairs allowing for more accurate detection and quantitation of the 15 +1 EU priority PAHs.

Another set of challenging analytes is the four dibenzopyrene isomers. Due to their high molecular weight (MW 302), these isomers are prone to discrimination and poor peak shape. Broad, tailing peaks make reliable quantitation difficult and decrease the signal to noise ratio, resulting in an increase in limits of detection. Deleterious chromatographic effects can be largely offset by limiting analyte dwell time on the column and in the GC/MS interface. Shorter columns with thinner film thickness and high operating temperatures are all factors that collectively can improve peak shapes for these analytes.

A column comparison study was done to evaluate column performance of a DB-EUPAH column versus a Restek's Rxi-17 column. Since the competitor does not offer this column in a 0.14 µm film thickness, a 20 m × 0.18 mm × 0.18 µm column was the closest available for the evaluation. A standard containing the 15+1 EU priority PAHs was analyzed on each column to evaluate performance.

Experimental

An Agilent 6890N Network GC system and Agilent 5975B Series MSD equipped with an Agilent 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for the analyses, and Table 2 lists the flow path consumable supplies.

Table 1. Chromatographic Conditions for 15+1 EU Priority PAHs Standards

GC:	Agilent 6890N/5975B MSD
Sampler:	Agilent 7683B, 5.0 µL syringe (Agilent p/n 5181-1273) 0.5 µL splitless injection, injection speed 75 µL/min
Carrier:	Helium, ramped flow 1.0 mL/min (0.2 min), 5 mL/min^2 to 1.7 mL/min
Inlet:	325 °C splitless, purge flow 60 mL/min at 0.8 min
Inlet Liner:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column :	Agilent J&W DB-EUPAH 20 m × 0.18 mm × 0.14 µm (Agilent p/n 121-9627)
Oven:	45 °C (0.8 min) to 200 °C (45 °C/min), 2.5 °C/min to 225 °C, 3 °C/min to 266 °C, 5 °C/min to 300 °C, 10 °C/min to 320 °C (4.5 min)
Detection:	MSD source at 300 °C, quadrupole at 180 °C, transfer line at 330 °C, Scan range 50–550 AMU
Column Performance Comparison	
Column 1:	Agilent J&W DB-EUPAH 20 m x 0.18 mm x 0.14 µm (Agilent p/n 121-9627)
Column 2:	Restek Rxi-17 20 m × 0.18 mm × 0.18 µm
Oven:	45 °C (0.8 min) to 200 °C (45 °C/min), 2.5 °C/min to 225 °C, 3 °C/min to 266 °C, 5 °C/min to 300 °C, 10 °C/min to 320 °C (4.5 min)

Table 2 Flow Path Supplies

Vials:	Amber crimp top glass vials (Agilent p/n 5183-4496)
Vial Caps:	Crimp caps (Agilent p/n 5181-1210)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; graphite (Agilent p/n 500-2114) 0.4 mm id short; preconditioned 85/15 Vespel/graphite, long (Agilent p/n 5062-3508)
20x magnifier:	20x Magnifier loop (Agilent p/n 430-1020)

Sample Preparation

A sixteen component EU PAH standard mix (Agilent p/n 5190-0487) served as a stock standard with a nominal concentration of 250 µg/mL per analyte. The working level standards were prepared with concentrations of 10, 5, 2, 1, 0.5, 0.2, and 0.1 µg/mL. All solutions were prepared in acetone using class A volumetric pipettes and flasks. JT Baker UltraResi grade acetone was used as a reagent blank and syringe wash solvent.

Results and Discussion

The 16-component EU regulated PAH standard mix was evaluated on an Agilent J&W DB-EUPAH 20 m × 0.18 mm × 0.14 µm (Agilent p/n 121-9627). Resolution of all sixteen PAHs was achieved in less than 43 min. An example chromatogram of the 2.5-ng on-column loading of the standard solution is shown in Figure 1.

Baseline resolution was achieved on the DB-EUPAH column for all of the analytes included in the 15 +1 EU PAH samples including three sets of critical pairs. Figure 1 highlights the resolution factors (R_s) for the three critical resolution pairs as calculated by the following formula [9]:

$$R_s = 1.18 * \frac{(T_{R2} - T_{R1})}{(W_{h1} + W_{h2})}$$

Where T_{R1} = retention time of the first peak
T_{R1} = retention time of the second peak
W_{h1} = peak width at half height of the first peak
W_{h2} = peak width at half height of the second peak

Baseline resolution between benz[a]anthracene and cyclopenta[c,d]pyrene was achieved with an R_s of 2.7 and R_s value of 2.4 for cyclopenta[c,d]pyrene and chrysene. Benzo[j]fluoranthene is normally difficult to separate from the [b] and [k] isomers; therefore these analytes are frequently reported as a sum. However, baseline resolution was achieved for these challenging isomers with the DB-EUPAH column with Rs values of 1.7 and 1.6, respectively, allowing accurate quantitation of each isomer. A resolution value of 2.6 was obtained for the third critical pair, indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene. Figure 1 clearly demonstrates that the DB-EUPAH column can provide excellent sensitivity and selectivity for the analysis of EU regulated PAHs.

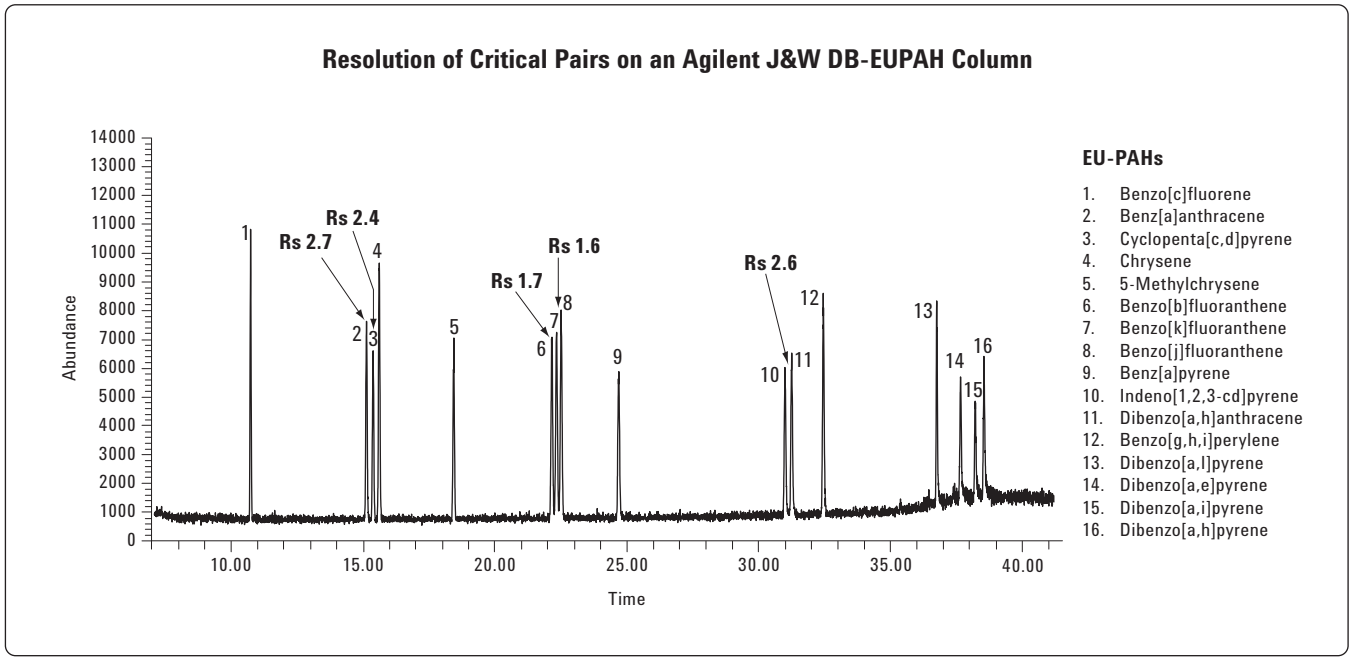

Figure 1. TIC of a 0.5 µL injection of 0.5 ng EU priority PAH standard solution on an Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm capillary GC column (Agilent p/n 121-9672). Chromatographic conditions are listed in Table 1.

For the comparison study, columns from each vendor were analyzed under the same conditions on the same instrument. Example total ion chromatograms (TIC) of 2.5-ng on-column loading for both vendor columns are shown in Figure 2. While both columns were able to resolve the EU PAHs under the same conditions, the DB-EUPAH did so with a significantly lower bleed profile.

The oven temperature program maximum was kept at 320 °C to accommodate the isothermal limit of the Rxi-17 column, during the comparison study. The maximum isothermal temperature limit for the DB-EUPAH column is 340 °C, 20 °C higher. The Rxi-17 column shows significantly higher bleed than the DB-EUPAH even at 320 °C, resulting in poorer signal-to-noise ratios as shown in Figure 3.

Figure 3 further illustrates the DB-EUPAH's superior sensitivity for the dibenzopyrenes at low levels by highlighting the signal-to-noise ratios of dibenzo[a,l]pyrene on both columns. The heavier dibenzopyrene isomers, in particular the [a,e], [a,i] and [a,h] isomers, were at the limit of reliable detection on the Rxi-17 column as a result of the higher bleed. Signal-to-noise ratios on the DB-EUPAH column are more than a factor of two better than those for the Rxi-17 because of its lower bleed.

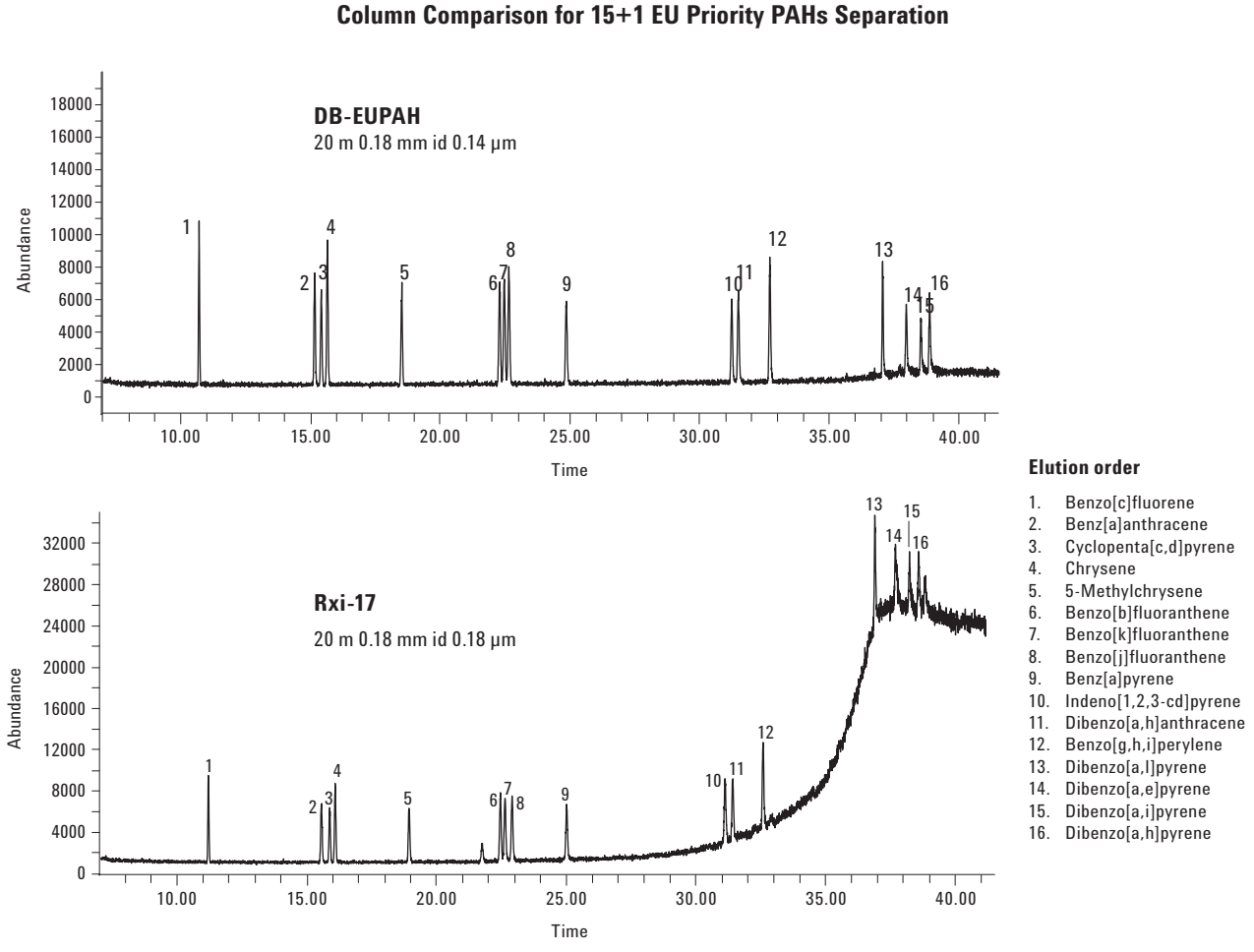

Figure 2. TIC of a 0.5 µL injection of the 0.5 µg/mL 15+1 EU priority PAH standard on Agilent J&W DB-EUPAH and Rxi-17 columns. Chromatographic conditions are listed in Table 1.

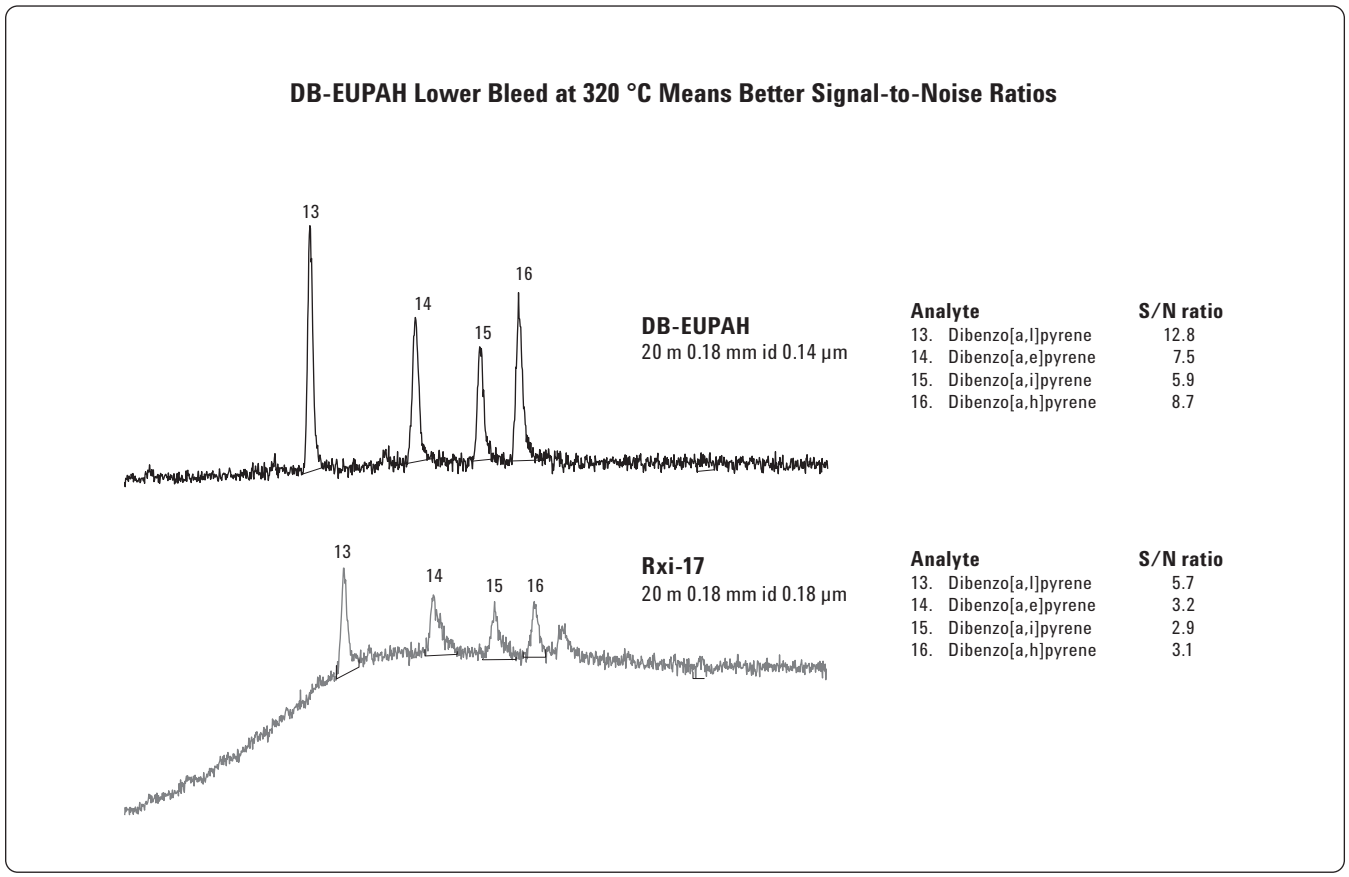

Figure 3. Overlaid TICs of 2.5 ng on-column loading injections of 15+1 EU priority PAH standard on Agilent J&W DB-EUPAH and Rxi-17. Chromatographic conditions are listed in Table 1.

Conclusions

This application demonstrates the use of an Agilent J&W DB-EUPAH capillary GC column for the analysis of 15+1 EU regulated PAHs. The 20 m × 0.18 mm, 0.14 µm column dimension effectively separated the sixteen PAHs, resolving all the critical, difficult-to-separate pairs. The column offers further improvement on the quantitation of the heavier dibenzopyrenes due to the higher temperature limit and phase ratio as compared to the Restek Rxi-17 column. Excellent peak shapes and sensitivity were achieved for the target 15 +1 EU priority PAHs.

The results of a column performance comparison between Agilent J&W DB-EUPAH column and the Rxi-17 showed lower bleed for the DB-EUPAH column with better signal to noise ratios for late eluting dibenzopyrene isomers. The excess bleed for the Rxi-17 column at the higher temperatures make trace level detection difficult and unreliable for the four dibenzopyrene isomers. The DB-EUPAH column offers improved peak shape and sensitivity that translates to consistently lower detection limits, which makes it an excellent choice for the analysis of the EU priority PAHs.

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