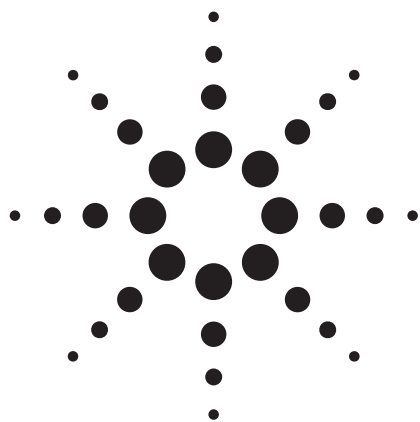




使用高效气相色谱柱分析 PAH： 色谱柱的选择与最佳实践

Food Quality and Environmental

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Abstract

欧盟（EU）对一系列主要在食品基质中发现的多环芳烃（PAH）进行了管制。此列表被称为 EU 15 +1 列表。美国国家环境保护局（USEPA）对一系列历史上被称作环境污染物的 16 种 PAH 具有管制规定。两个列表包含了不同的分析物，带来不同的分离挑战。但两张列表中有 8 种分析物是相同的。在本研究中，采用安捷伦 J&W DB-EUPAH 20 m×0.18 mm, 0.14 μm 高效气相色谱柱，在 28 分钟内实现了规定的所有 24 种受管制 PAH 的分离。使用安捷伦 J&W DB-5ms 20 m×0.18 mm, 0.18 μm 高效气相色谱柱在 22 分钟内分离出了 24 种受管制 PAH 中的 23 种。安捷伦 J&W DB-EUPAH 和 DB-5ms 色谱柱二者均为受管制 PAH 分析的最佳选择。建议将安捷伦 J&W DB-EUPAH 用于苯并[b,j,k] 荧蒽异构体的分离。



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Instruction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings. PAHs often result from the incomplete combustion of organic substances such as wood, coal, and oil. The European Union PAH regulation has focused on these substances as potential contaminants in the food supply. A main source of potential human exposure to PAHs is through heat processing of meat and dairy products, such as grilling and smoking [1]. There are serious health concerns regarding PAHs since many are classified as carcinogenic or mutagenic [2].

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) [3]. The EU 15+1 priority PAHs along with the US-EPA-regulated PAHs are provided in Table 1 [4]. There are eight PAHs that are common to both the EU-15 +1 list and the US-EPA list.

A 5% phenyl methylpolysiloxane stationary phase column is the most commonly used GC column for PAH analysis. This nonpolar column yields good resolution for the 16 US-EPA PAHs [5,6], however, three critical pairs of the 15+1 EU PAHs co-elute and are difficult to resolve by mass spectrometry [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 the limits of detection. Limiting analyte dwell time on the column and in the GC/MS interface can offset these deleterious chromatographic effects. Shorter columns with thinner film thickness and high operating temperatures are all factors that collectively can improve peak shapes for these analytes.

Table 1. EU and US-EPA Regulated PAH Compounds

Peak #	Component	CAS #	MW	EU 15+1	EPA
1	Naphthalene	91-20-3	128		x
2	Acenaphthylene	208-96-8	152		x
3	Acenaphthene	83-32-9	154		x
4	Fluorene	86-73-7	166		x
5	Phenanthrene	85-01-8	178		x
6	Anthracene	120-12-7	178		x
7	Fluoranthene	206-44-0	202		x
8	Pyrene	129-00-0	202		x
9	<i>Benzo[c]fluorene</i>	205-12-9	216	x	
10	Benz[a]anthracene	56-55-3	228	x	x
11	<i>Cyclopenta[c,d]pyrene</i>	27208-37-3	226	x	
12	Chrysene	218-01-9	228	x	x
13	<i>5-Methylchrysene</i>	3697-24-3	242	x	
14	Benzo[b]fluoranthene	205-99-2	252	x	x
15	Benzo[k]fluoranthene	207-08-9	252	x	x
16	<i>Benzo[j]fluoranthene</i>	205-82-3	252	x	
17	Benzo[a]pyrene	50-32-8	252	x	x
18	Indeno[1,2,3-cd]pyrene	193-39-5	276	x	x
19	Dibenz[a,h]anthracene	53-70-3	278	x	x
20	Benzo[g,h,i]perylene	191-24-2	276	x	x
21	<i>Dibenzo[a,l]pyrene</i>	191-30-0	302	x	
22	<i>Dibenzo[a,e]pyrene</i>	192-65-4	302	x	
23	<i>Dibenzo[a,i]pyrene</i>	189-55-9	302	x	
24	<i>Dibenzo[a,h]pyrene</i>	189-64-0	302	x	

Regulated PAH compounds shown in plain text are included only in the US-EPA set, compounds in *Italic* are included only in the EU 15+1 list, and the compounds in **bold** are included in both the US-EPA and EU 15+1 lists.

Experimental

GC EU PAH standard (Agilent p/n 5190-0487) and US-EPA mixture (Agilent p/n 8500-6035) were diluted separately to a concentration of 2 µg/ml using class A glassware and pipets. These solutions were then mixed 1:1 to for a final concentration of 1-2 µg/ml of all 24 regulated PAHs.

Table 2. Chromatographic Conditions DB-EUPAH Column

Sample:	0.5 µL 1-2 µg/mL EU + EPA PAH combined standards (EU PAH standard Agilent p/n 5190-0487 and EPA PAH standard Agilent p/n 8500-6035)
GC/MS:	Agilent 7890A GC System with an Agilent 5975C Series GC/MSD, TAD, and an Agilent 7873B automatic liquid sampler
Column:	Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm (Agilent p/n 121-9627)
Carrier:	Helium 60 cm/sec 1.8 ml/min constant flow
Oven:	70 °C (0.8 min), 70 °C/min to 180 °C, 7 °C/min to 230 °C (6 min), 40 °C/min to 280 °C (5 min), 25 °C/min to 335 °C (5 min)
Inlet:	300° C splitless, purge 100 mL/min at 0.25 min
Inlet liner:	Helix double taper deactivated (Agilent p/n 5188-5398)
MSD:	Sim/Scan mode 50-400 AMU, transfer line 340 °C, source 340 °C, quad 150 °C

Table 3. Chromatographic Conditions DB-5ms Column

Sample:	0.5 µL 1-2 µg/mL EU + EPA PAH combined standards (EU PAH standard Agilent part # 5190-0487 and EPA PAH standard Agilent part # 8500-6035)
GC/MS:	Agilent 7890A GC System with an Agilent 5975C Series GC/MSD, TAD, and an Agilent 7873B automatic liquid sampler
Column:	Agilent J&W DB-5 ms 20 m × 0.18 mm, 0.18 µm (Agilent p/n 121-5522)
Carrier:	Helium 60 cm/sec 1.8 ml/min constant flow
Oven:	55 °C (0.4 min), 25 °C/min to 200 °C, 8 °C/min to 280 °C, 10 °C/min to 320 °C (2 min), 25 °C/min to 335 °C (5 min)
Inlet:	300 °C splitless, purge 100 mL/min at 0.25 min
Inlet liner:	Helix double taper deactivated (Agilent part # 5188-5398)
MSD:	Sim/Scan mode 50-400 AMU, transfer line 340 °C, source 340 °C, quad 150 °C

Table 4. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial Caps:	Screw caps (Agilent p/n 5182-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5183-4729)
Septum:	Advanced green (Agilent p/n 5183-4759)
Inlet Seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
Inlet liners:	Helix double taper deactivated (Agilent p/n 5188-5398)
Ferrules:	0.4 mm ID short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
20 × magnifier :	20 × Magnifier loop (Agilent p/n 430-1020)

Discussion of Results

Figure 1 shows the separation of all 24 analytes included in the EU 15 + 1 and US-EPA PAH lists on a DB-EUPAH column. This separation was accomplished in under 28 minutes on an Agilent J&W DB-EUPAH 20 m x 0.18 mm, 0.14 μ m column (Agilent p/n 121-9627). The selectivity of this midpolar column is necessary to resolve the benzo(b,j,k) fluoranthene isomers. High temperature stability is also required for elution of the high boiling point dibenzopyrenes.

The injection volume was reduced to 0.5 μ L in order to scale the separation to the 0.18 or high efficiency GC (HEGC) format. This is often a necessary step when working with high efficiency columns because the higher efficiency of narrow bore columns is at the expense of sample loading capacity. Here the separation focused on achieving faster analysis. An example of a separation maximizing resolution with longer retention on this column was described previously [9].

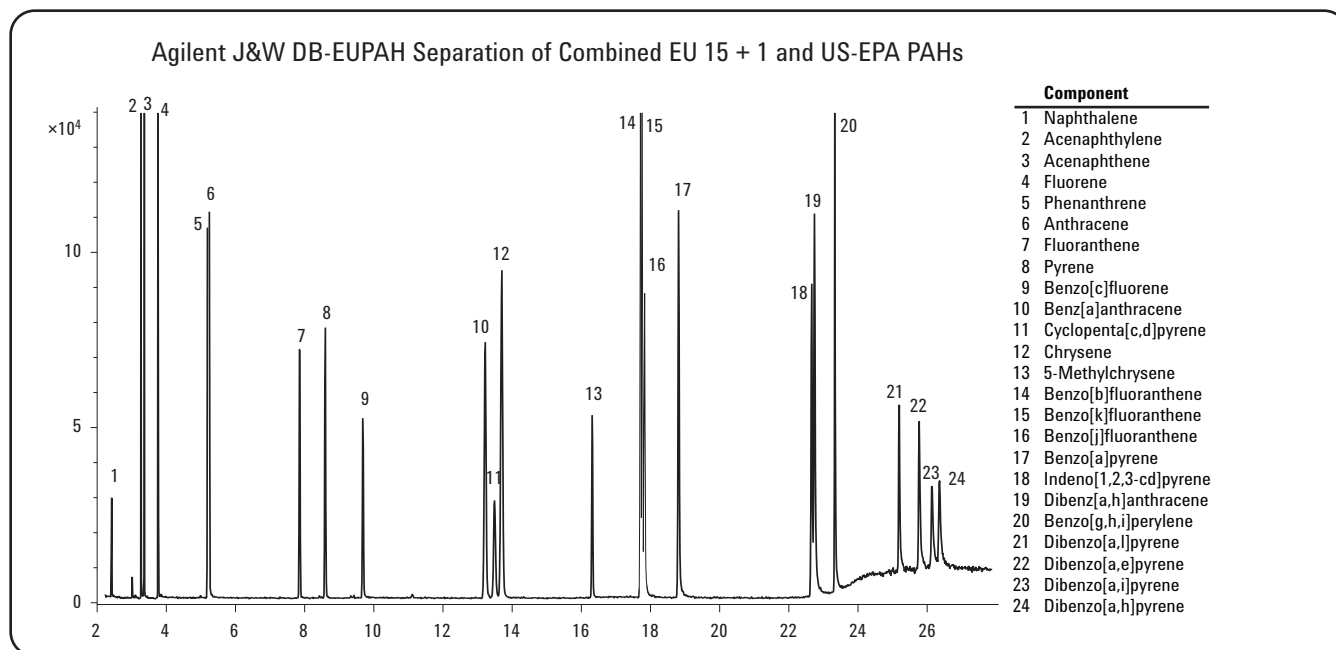


Figure 1. EU and US-EPA regulated PAH separation on an Agilent J&W DB-EUPAH 20 m x 0.18 mm, 0.14 μ m column (Agilent p/n 121-9627). Chromatographic conditions as in Table 2 and flow path supplies as in Table 4.

Figure 2 shows the resolution of 23 of the 24 analytes included in the EU 15 + 1 and US-EPA PAH lists on an Agilent J&W DB-5ms column. Benzo[j] fluoranthene is not resolved from benzo[k]fluoranthene using this column. However, when it is sufficient to report the sum of the benzofluoranthene isomers, the Agilent J&W DB-5ms column is an excellent choice for the 24 regulated PAHs. This separation was accomplished in under 22 minutes on an Agilent J&W DB-5ms 20 m × 0.18 mm, 0.18 µm column (Agilent p/n 121-5522). The Agilent J&W DB-5ms separation offers a 27 % faster analysis time when compared to the DB-EUPAH separation shown in Figure 1.

There are a number of best practices to consider when optimizing a GC/MS system for PAH analysis. The use of retention gaps and/or inlet backflushing can reduce maintenance and cycle times. Close examination of injection parameters such as injection volume, inlet temperature, purge time activation, solvent focusing and holding the oven temperature stable during injection can all contribute to better results. Minimizing inlet and system dwell time by operating at high linear velocities can also improve results. Another best practice for PAHs is to keep heated zones well insulated and hot to reduce the potential for system cold spots and the resultant signal loss.

Conclusions

All 24 (EU 15 + 1 and US-EPA) regulated PAHs are resolved using an Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm column. The benzo[b,j,k]fluoranthene isomers were adequately separated for individual quantitation. This is the column of choice when resolution of benzo[j]fluoranthene is required. This separation was accomplished in under 28 minutes.

23 of 24 (EU 15 + 1 and US-EPA) regulated PAHs resolve using an Agilent J&W DB-5ms 20 m × 0.18 mm, 0.18 µm column. This column is an excellent choice when benzofluoranthene isomers are reported as a sum of the isomers and speed of analysis is critical. This separation was accomplished in under 22 minutes.

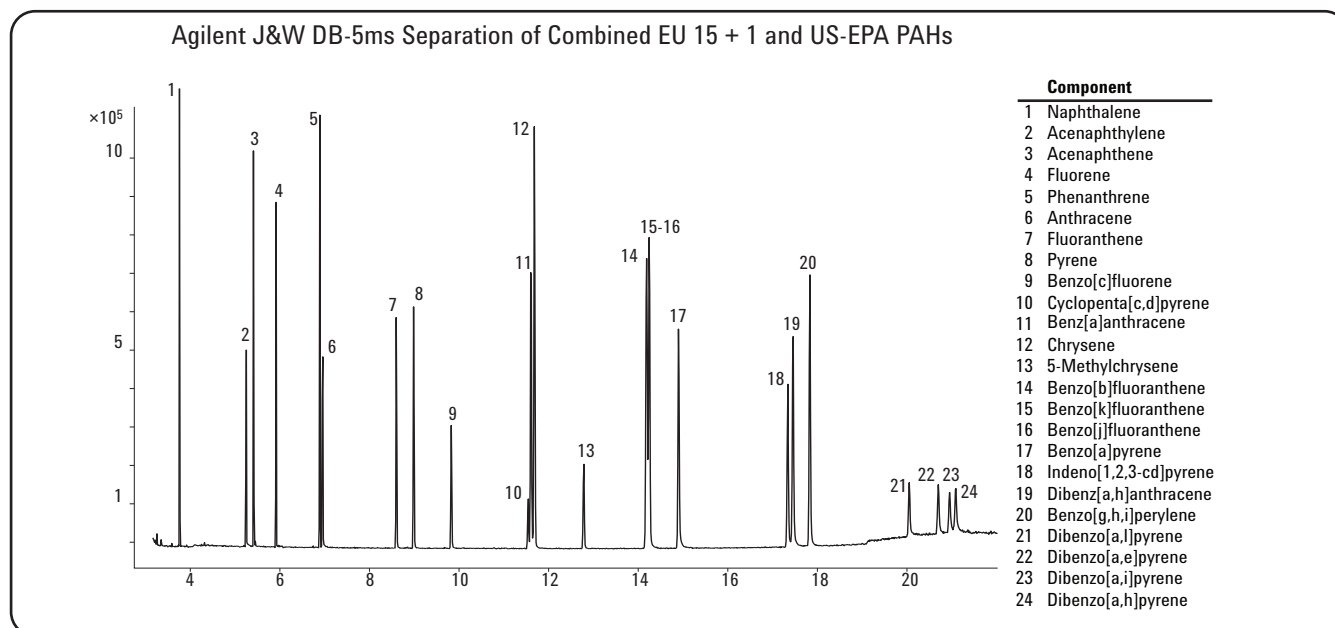


Figure 2. EU and US-EPA regulated PAH separation on an Agilent J&W DB-5 ms 20 × 0.18 mm, 0.18 µm (Agilent p/n 121-5522) column. Chromatographic conditions as in Table 3 and flow path supplies as in Table 4.

Factors to consider in optimizing EU 15 +1 and US-EPA PAH analyses

- Choose an Agilent J&W DB-EUPAH when the resolution of benzo[b,j,k]fluoranthene isomers (24 of 24 peaks in combined set) is required.
- Choose an Agilent J&W DB-5ms when benzo[b,j,k]fluoranthene isomers can be reported as a sum of the isomers. The Agilent J&W DB-5ms resolves 23 of 24 regulated PAHs in 27 % faster cycle time than the Agilent J&W DB-EUPAH column.
- Consider the use of retention gaps and inlet backflushing to reduce cycle time and maintenance.
- Achieve faster analysis times with no loss of resolution using 0.18 mm id high efficiency GC columns.
- Optimize injection volume, temperature, purge time, and solvent focusing for best results on your instrument.
- Minimize inlet and system dwell time with high linear velocities.
- Keep heated zones hot to avoid cold spots and signal loss.

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