APPLICATIONS

Rapid GC-MS Analysis of European Food Safety Agency (EFSA) PAH Compounds in Food using a Zebron™ ZB-PAH-EU GC Column

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic substances that must be monitored in food products per guidelines set forth by the European Food Safety Agency (EFSA) prior to distribution within the European Union. PAH compounds may arise in meat and dairy products upon heat processing, during which amino acid residues in food may react with sugars and creatine to generate PAHs. In addition, packaging materials in food may also introduce PAH components. The EFSA has identified four PAHs as the "EFSA PAH4," which consists of benzo[a]pyrene, benz[a]anthracene, chrysene, and benzo[b]fluoranthene. These four PAH compounds are representative of the carcinogenic characteristic of most food samples. It is very important to quantitate these 4 PAHs in food without any false positives from other interfering PAH components. Also, it is essential to analyze these 4 PAHs in the shortest run time to improve the throughput of this analysis. In this study, the optimal selectivity of the Zebron ZB-PAH-EU capillary GC column is utilized to separate "EFSA PAH4" compounds in a short run time of less than 10 minutes. In addition, a 16 component EU (15+1) mixture was injected to confirm that there were no interferences with the "EFSA PAH4" test compounds, ensuring accurate quantification of food samples.

Figure 1a.
Separation of "EFSA PAH4" on a 30 m x 0.25 mm x 0.20 µm Zebron ZB-PAH-EU GC Column

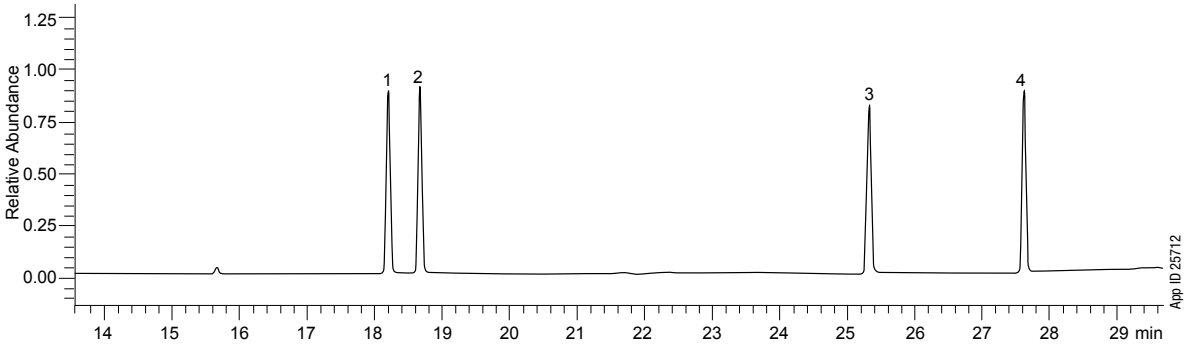
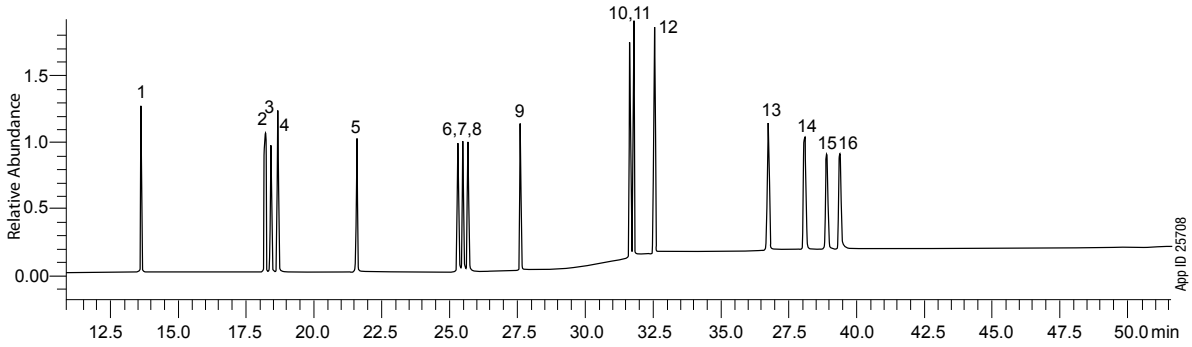


Figure 1b.
Separation of Interfering PAHs (EU 15+1) on a 30 m x 0.25 mm x 0.20 µm Zebron ZB-PAH-EU GC Column



Column: Zebron ZB-PAH-EU
Dimensions: 30 meter x 0.25 mm x 0.20 µm
Part No.: 7HG-G043-10
Inlet Liner: AG2-3B03-05
Injection: Split (5:1) @ 330 °C, 1 µL
Carrier Gas: Helium @ 24 psi (constant pressure)
Oven Program: 45 °C for 0.8 min to 200 °C @ 45 °C/min to 226 °C @ 3 °C/min to 320 °C @ 10 °C/ min for 20 min
Detector: MSD (Shimadzu® GC-MS-QP2010 Ultra)
Transfer Line Temperature 300 °C
Source Temperature: 300 °C
Mode: Scan (50-500 m/z)

Sample (Figure 1a):10 ppm "EFSA PAH4" Standard in Toluene

Analyte Name	RT (min)
1. Benz[a]anthracene	18.25
2. Chrysene	18.75
3. Benzo[b]fluoranthene	25.32
4. Benzo[a]pyrene	27.60

Sample (Figure 1b): 10 ppm EU (15+1) PAH Standard in Toluene

Analyte Name	RT (min)
1. Benzo[c]fluorene	13.60
2. Benz[a]anthracene	18.25
3. Cyclopenta[c,d]pyrene	18.45
4. Chrysene	18.60
5. 5-Methylchrysene	21.50
6. Benzo[b]fluoranthene	25.30
7. Benzo[k]fluoranthene	25.45
8. Benzo[j]fluoranthene	25.65
9. Benzo[a]pyrene	27.60
10. Indeno[1,2,3-cd]pyrene	31.60
11. Dibenz[a,h]anthracene	31.75
12. Benzo[g,h,i]perylene	32.55
13. Dibenzo[a,l]pyrene	36.75
14. Dibenzo[a,e]pyrene	38.00
15. Dibenzo[a,i]pyrene	38.89
16. Dibenzo[a,h]pyrene	39.30

Figure 2a.
Separation of "EFSA PAH4" on a 20 m x 0.18 mm x 0.14 µm Zebron™ ZB-PAH-EU GC Column

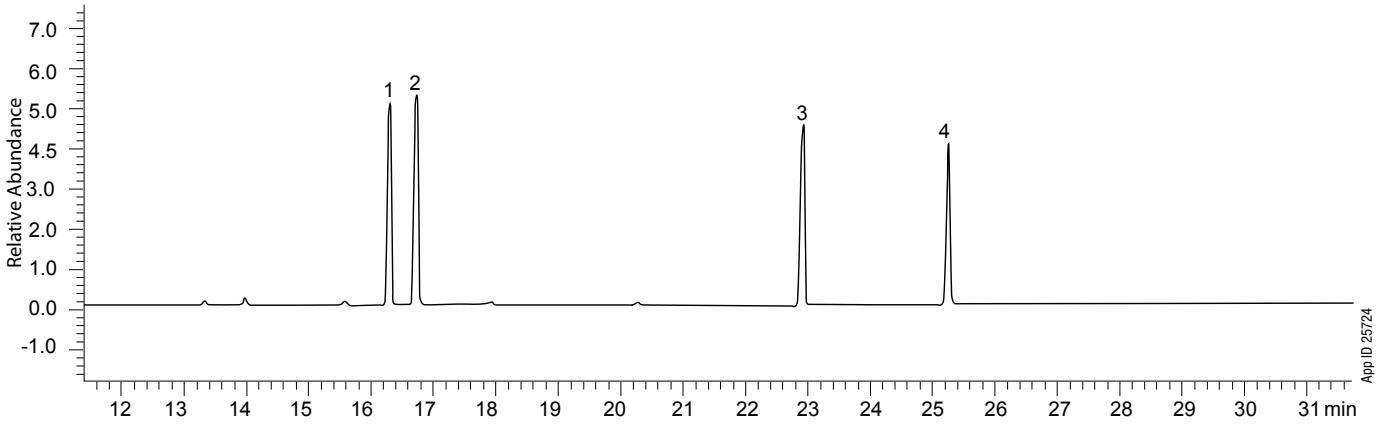
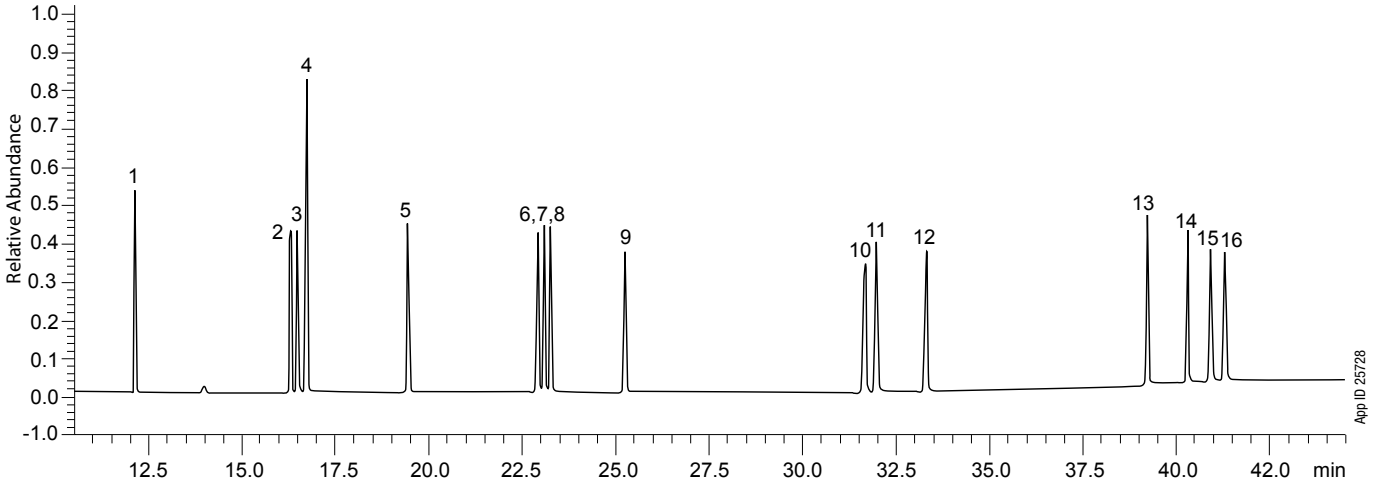


Figure 2b.
Separation of Interfering PAHs (EU 15+1) on a 20 m x 0.18 mm x 0.14 µm Zebron ZB-PAH-EU GC Column



Column: Zebron ZB-PAH-EU
Dimensions: 20 meter x 0.18 mm x 0.14 µm
Part No.: 7FD-G043-47
Inlet Liner: AG2-3B03-05
Injection: Split (5:1) @ 330 °C, 1 µL
Carrier Gas: Helium @ 1.0 mL/min (constant flow)
Oven Program: 45 °C for 0.8 min to 200 °C @ 45 °C/min to 300 °C @ 10 °C/min to 320 °C for 5 min
Detector: MSD (Shimadzu® GC-MS-QP2010 Ultra)
Transfer Line Temperature 300 °C
Source Temperature: 300 °C
Mode: Scan (50-500 m/z)

Sample (Figure 2a): 10 ppm "EFSA PAH4" Standard in Toluene

Analyte Name	RT (min)
1. Benz[a]anthracene	16.30
2. Chrysene	16.70
3. Benzo[b]fluoranthene	22.90
4. Benzo[a]pyrene	25.26

Sample (Figure 2b): 10 ppm EU (15+1) PAH Standard in Toluene

Analyte Name	RT (min)
1. Benzo[c]fluorene	12.12
2. Benz[a]anthracene	16.30
3. Cyclopenta[c,d]pyrene	16.50
4. Chrysene	16.75
5. 5-Methylchrysene	19.45
6. Benzo[b]fluoranthene	22.95
7. Benzo[k]fluoranthene	23.10
8. Benzo[j]fluoranthene	23.26
9. Benzo[a]pyrene	25.25
10. Indeno[1,2,3-cd]pyrene	31.65
11. Dibenz[a,h]anthracene	31.97
12. Benzo[g,h,i]perylene	33.30
13. Dibenzo[a,l]pyrene	39.22
14. Dibenzo[a,e]pyrene	40.30
15. Dibenzo[a,i]pyrene	40.92
16. Dibenzo[a,h]pyrene	41.33

Figure 3a.
Fast Separation of "EFSA PAH4" on a 10 m x 0.10 mm x 0.08 µm Zebron™ ZB-PAH-EU GC Column

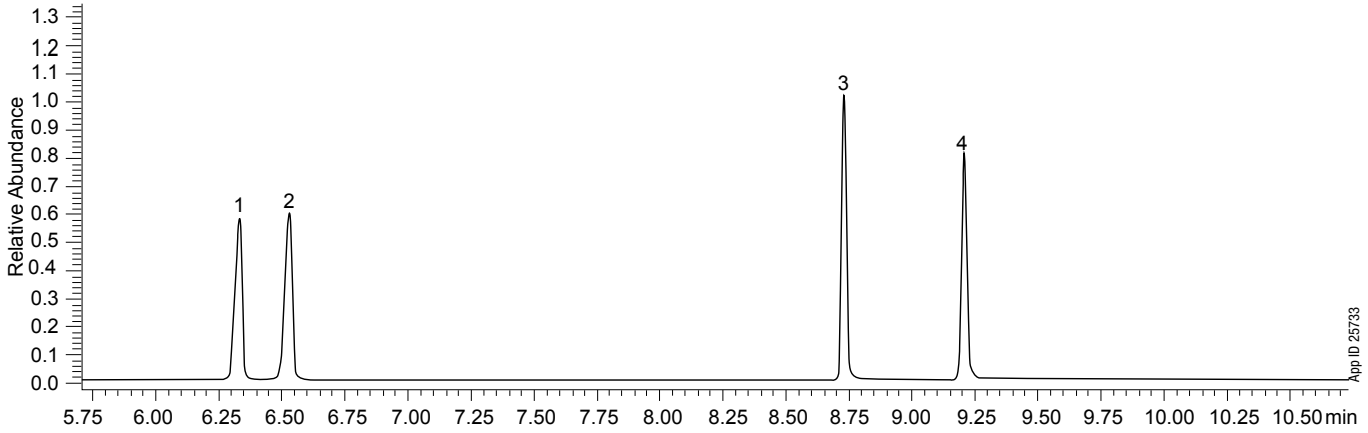
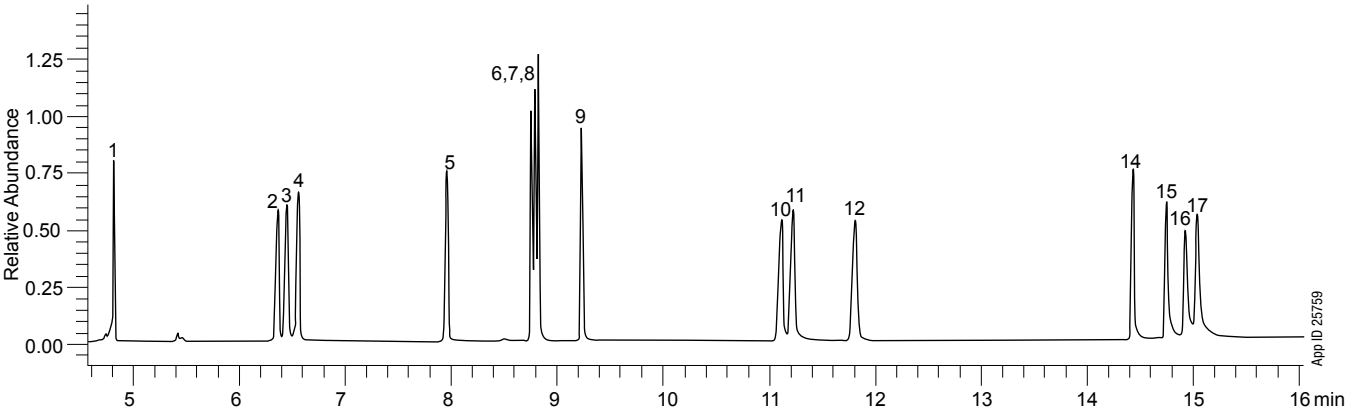


Figure 3b.
Separation of Interfering PAHs (EU 15+1) on a 10 m x 0.10 mm x 0.08 µm Zebron ZB-PAH-EU GC Column



Column: Zebron ZB-PAH-EU
Dimensions: 10 meter x 0.10 mm x 0.08 µm
Part No.: 7CB-G043-59
Inlet Liner: AG2-3B03-05
Injection: Split (15:1) @ 320 °C, 1 µL
Carrier Gas: Helium @ 0.88 mL/min (constant Flow)
Oven Program: 70 °C for 0.4 min to 180 °C @ 140 °C/min to 230 °C @ 14 °C/ min for 3 min to 280 °C @ 85 °C/ min for 5 min to 330 °C @ 40 °C/min for 5 min
Detector: MSD (Shimadzu® GC-MS-QP2010 Ultra)
Transfer Line Temperature: 300 °C
Source Temperature: 300 °C
Mode: Scan (100-500 m/z)

Sample (Figure 3a): 10 ppm "EFSA PAH4" Standard in Toluene

Analyte Name	RT (min)
1. Benz[a]anthracene	6.27
2. Chrysene	6.52
3. Benzo[b]fluoranthene	8.75
4. Benzo[a]pyrene	9.20

Sample (Figure 3b): 10 ppm EU (15+1) PAH Standards in Toluene

Analyte Name	RT (min)
1. Benzo[c]fluorene	4.82
2. Benz[a]anthracene	6.27
3. Cyclopenta[c,d]pyrene	6.45
4. Chrysene	6.52
5. 5-Methylchrysene	7.95
6. Benzo[b]fluoranthene	8.75
7. Benzo[k]fluoranthene	8.80
8. Benzo[j]fluoranthene	8.82
9. Benzo[a]pyrene	9.20
10. Indeno[1,2,3-cd]pyrene	11.10
11. Dibenz[a,h]anthracene	11.23
12. Benzo[g,h,i]perylene	11.80
13. Dibenzo[a,l]pyrene	14.40
14. Dibenzo[a,e]pyrene	14.70
15. Dibenzo[a,i]pyrene	14.92
16. Dibenzo[a,h]pyrene	15.05

Table 1.
Resolution of "EFSA PAH4" from Interfering PAHs

Analyte	30 m Zebron ZB-PAH-EU (Figure 1b)	20 m Zebron ZB-PAH-EU (Figure 2b)	10 m Zebron ZB-PAH-EU (Figure 3b)
Benz[a]anthracene and Cyclopenta[c,d]pyrene	2.2	2.0	1.4
Cyclopenta[c,d]pyrene and Chrysene	2.2	2.2	1.8
Benzo[b]fluoranthene and Benzo[k]fluoranthene	1.5	1.5	1.0
Benzo[j]fluoranthene and Benzo[a]pyrene	17.7	17.3	11.1

Results and Discussion

The Zebron ZB-PAH-EU GC column offers unique selectivity for the separation of closely related PAHs. The PAH compounds of interest within the "EFSA PAH4" standard are fully resolved from each other as well as from other components of the "EU 15+1" standard mixture. **Figure 1a** shows the separation of "EFSA PAH4" compounds Benz[a]anthracene, Chrysene, Benzo[b]fluoreanthene and Benzo[a]pyrene baseline resolved from each other on a Zebron ZB-PAH-EU 30 m x 0.25 mm x 0.20 µm GC column. The proprietary stationary phase selectivity of the Zebron ZB-PAH-EU GC column affords the complete resolution of critical pairs while the optimal dimension offers high efficiency. The key interferences were investigated by injecting EU (15+1) PAHs. Benz[a]anthracene is resolved completely from Cyclopenta[c,d]pyrene; Benzo[b]fluoranthene is likewise resolved from both Benzo[k]fluoranthene and Benzo[j]fluoranthene. Benzo[a]pyrene is clearly resolved from nearby PAHs as shown in **Figure 1b**. The resolution values for critical interfering PAHs are presented in **Table 1**.

The column dimensions, 30 m x 0.25 mm x 0.20 µm, completely resolve all the components of the "EU 15+1" standard profile. The critical pairs of Indeno[1,2,3-cd]pyrene and Dibenzo[a,h]anthracene are resolved later in the analysis during a temperature hold of 320 °C. The stationary phase of the Zebron ZB-PAH-EU is reinforced which entails the Engineering Self Cross-linking (ESC™), through extensive crosslinking of pure stationary phase film. Hence, the stationary-phase is then able to withstand exposure to higher temperatures, allowing the column a temperature rating of 340 °C/360 °C, and seamless elution and bakeout when evaluating high-boiling PAH substances. Such high temperature limits are extremely helpful for easy bakeout of dirty matrices such as food that can otherwise produce ghost peaks.

A 20 or 10 meter Zebron ZB-PAH-EU GC column offers faster analysis for high throughput applications. **Figure 2a** represents the "EFSA PAH4" separation on a 20 meter column and **Figure 2b** confirms the separation of interfering components. Similarly, **Figures 3a** and **3b** represent the separation of EFSA PAH4 and interfering PAH components using a shorter, 10 meter column. As shown in **Figure 3a**, the run time can go as low as 10 minutes. This is a 3-fold increase in speed of analysis as compared to a 30 meter column. Such fast analysis is very useful laboratories labs that need to run multiple samples. With closely matching phase ratios among dimensions of the Zebron ZB-PAH-EU, seamless transfer of analysis can be achieved to accomplish a high throughput analysis. In addition, the proprietary selectivity and efficiency of the Zebron ZB-PAH-EU GC column enhances the resolution of critical PAH compounds.

Conclusion

One of the main objectives of this technical note is to demonstrate the resolution of "EFSA PAH4" from EU (15+1) PAHs. The Zebron ZB-PAH-EU GC column provides a solution for separating the four most common PAH markers within "EFSA PAH4" for food analysis. The ZB-PAH-EU may also distinguish the components of the "EFSA PAH4" from the additional PAH compounds featured in the EU (15+1) PAH standard. The higher temperature limit of 340/360 °C is extremely helpful for baking out food matrix contaminants. Optimal dimensions and selectivity help to achieve a run time as short as 10 min for "EFSA PAH4" components.

Zebron™ ZB-PAH-EU Ordering Information

ID(mm)	df(µm)	Temp. Limits °C	Part No.
10-Meter			
0.10	0.08	40 to 340/360	7CB-G043-59
20-Meter			
0.18	0.14	40 to 340/360	7FD-G043-47
30-Meter			
0.25	0.20	40 to 340/360	7HG-G043-10
60-Meter			
0.25	0.20	40 to 340/360	7KG-G043-10

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