APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

**Authors**

**Meng Yuan**

**PerkinElmer, Inc.**
**Shelton, CT 06484 USA**

**Jie Jiang, Ting Mao, Liping Feng**

**Beijing Municipal**
**Center for Food Safety**
**Beijing, China**

# The Preparation and Analysis of Polycyclic Aromatic Hydrocarbons in Meat by GC/MS

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbon molecules containing two or more aromatic rings. Some PAHs, such as benzo[a]pyrene, are classified as carcinogens; PAHs are commonly found in the environment as a result of partially burned organic materials, such as petroleum, plastics, rubber, lubricants and wood. In addition to environmental concerns, there are concerns about PAHs in food, especially in grilled meats.

The European Union (EU) introduced legislation in early 2005 in response to food-contamination data collected by the Scientific Committee on Food (SCF). The SCF identified 15 PAHs that possess carcinogenic properties. Directive 2005/69/EC of the European Parliament and of the Council of 16 November 2005 identified an additional PAH as probably carcinogenic.[1] The joint set of PAHs was recognized as the 15 + 1 EU priority PAHs. It is necessary to accurately determine the level of 15 + 1 EU priority PAHs in food to respond to European legislation and ensure food safety.

This application note will present a method developed to measure 15 + 1 EU priority PAHs at low levels using gas chromatography mass spectrometry (GC/MS). It will also describe a reliable procedure for extraction and purification of PAHs from meat samples. The sample preparation will focus on benzo[a] pyrene. In addition to method optimization and calibration, a variety of meat samples are analyzed and the amount of PAHs determined.

PerkinElmer®
*For the Better*

## Experimental

### Solvent Extraction

A solvent extractor (Dionex® ASE 300) was used to extract the meat samples analyzed in this application. A glass microfiber filter was put at the outlet end of the extraction cells. Then 1 g of diatomaceous earth was put on top of the glass fiber filter. 5 g of homogenized-meat sample were mixed with 5 g of Florisil®. The resulting material was poured into the extraction cells. 2 g of anhydrous sodium sulfate was put into the collection vials to remove all water in the meat samples. Table 1 shows the instrumental setup parameters for the accelerated solvent extractor.

***Table 1.*** **Instrumental Parameters for the Solvent Extractor.**

| | |
|---|---|
| Solvent | Cyclohexane |
| Pressure of Nitrogen | 1400 psi |
| Extraction Temperature | 100 °C |
| Heat Time | 5 min |
| Static Time | 5 min |
| Cycles | 2 |
| Flush Volume | 60% |
| Purge Time | 100 sec |

### Gel Permeation Chromatography (GPC)

The extract was concentrated to 2 mL using a rotary evaporator at 35 °C and diluted quantitatively to 5 mL with cyclohexane:ethylacetate (50:50 v/v). It was then filtered through a PTFE filter (5 µm) to remove any particulates.

The GPC system was an AccuPrep™ MPS and AccuVap™ Inline with an Express™ GPC cleanup column from J2 Scientific. 2 mL of each sample was injected into GPC. Samples were eluted at a flow rate of 4.7 mL/min by cyclohexane:ethylacetate (50:50 v/v) (dump time 0–15 min, collect time 15–20 min).

The focus of the sample analysis, as mentioned in the introduction, was on benzo[a]pyrene. As a result, the collection time of the GPC began just before the elution of benzo[a] pyrene to eliminate as much matrix as possible. The timing of the fraction collection sent most of the co-extracted matrix to waste; however, it also resulted in the dumping of the 5 highest-molecular-weight PAHs in the mix. If these PAHs were of interest the GPC, collect time would be adjusted to collect all of the PAHs. Following fraction collection, the final volume was adjusted to 1.5 mL with cyclohexane.

### Standard Preparation

A stock solution at 20 µg/mL was prepared by diluting 0.2 mL of a 1000 µg/mL PAHs standard to 10 mL with cyclohexane.

1 µg/mL standard working solution was prepared by diluting 0.5 mL of a 20 µg/mL PAHs standard stock solution to 10 mL with cyclohexane.

An internal standard solution mixture of naphthalene-D8, phenanthrene-D10, chrysene-D12 and perylene-D12 at 20 ng/µL was prepared by diluting 0.2 mL of a 1000 µg/mL internal standard solution mixture to 10 mL with cyclohexane.

Working calibration standards at 5, 10, 20, 50, 100, 200, 500 ng/mL were prepared fresh each day. 5 µL of the 20 ng/µL internal standard solution was injected into each GC vial containing 1 mL of the working standard or sample.

### GC/MS Conditions

In this application, the PerkinElmer® Clarus® 680 GC/MS system was used to identify and quantify PAHs. Table 2 shows the detailed instrumental setup parameters for the GC/MS system.

***Table 2.*** **Instrumental Parameters.**

| **Gas Chromatograph** | **PerkinElmer Clarus 680 GC** |
|---|---|
| Oven Program Initial Temperature | 50 °C |
| Hold Time 1 | 2 min |
| Ramp 1 | 25 °C/min to 200 °C |
| Hold Time 2 | 0 min |
| Ramp 2 | 15 °C/min to 310 °C |
| Hold Time 3 | 19.67 min |
| Equilibration Time | 0.2 min |
| Column | PerkinElmer – Elite™-5ms 30 m x 0.25 mm x 0.25 µm |
| Injector | Programmable Split/Splitless |
| Injection Mode | Splitless and Pressure Pulse |
| Injection Volume | 1 µL |
| Inlet Temperature | 280 °C |
| Liner | Deactivated Liner (P/N N6502002) |
| Carrier Gas | Helium |
| Carrier Gas Flow Rate | 1 mL/min |
| **Mass Spectrometer** | **PerkinElmer Clarus 600 MS** |
| Mass Range | 45-450 u |
| Solvent Delay Time | 6 min |
| Scan Time | 0.20 sec |
| InterScan Delay Time | 0.05 sec |
| Transfer Line Temperature | 280 °C |
| Source Temperature | 240 °C |
| Mutiplier | 500 V |
| InterChannel Delay Time | 0.05 sec |
| SIM Mode | 8 SIM groups |
| SIR Dwell Time | 0.04 sec |
| Software | TurboMass™ 5.4.2 |

The regulatory limits for the analysis of benzo[a]pyrene require low-level analysis. In order to achieve this level of detection in sample matrix, it was necessary to use selected ion monitoring (SIM). The steps to create a SIM MS method follow:

- Each PAH had one quantifier ion and two qualifier ions (Table 3 – Page 4).
- The dwell time of each ion was 0.04 seconds.
- All isomers were in the same group. Other PAHs were in the separate groups.

## Results

The precision of the method was evaluated with a 100 ng/mL standard – Table 3 shows % RSD from this experiment. Figure 1 is an example chromatogram of a 200 ng/mL standard injection.

Chrysene-D12 and perylene-D12 were used as internal standards. Peak-area ratio was used to calculate amounts of PAHs.

The peak-area ratio for the compound in the sample was calculated by dividing the peak area of the compound (target ion) by the peak area (target ion) of the internal standard (IS):

$$Peak-area\ ratio = \frac{Peak\ area\ of\ the\ component\ ion}{Peak\ area\ of\ the\ IS\ ion}$$

Amounts of PAHs were calculated by plotting the peak-area ratio in the following calibration functions:

$$conc\ (x) = \frac{y(peak-area\ ratio) - b^{\,y=ax+b}}{a}$$

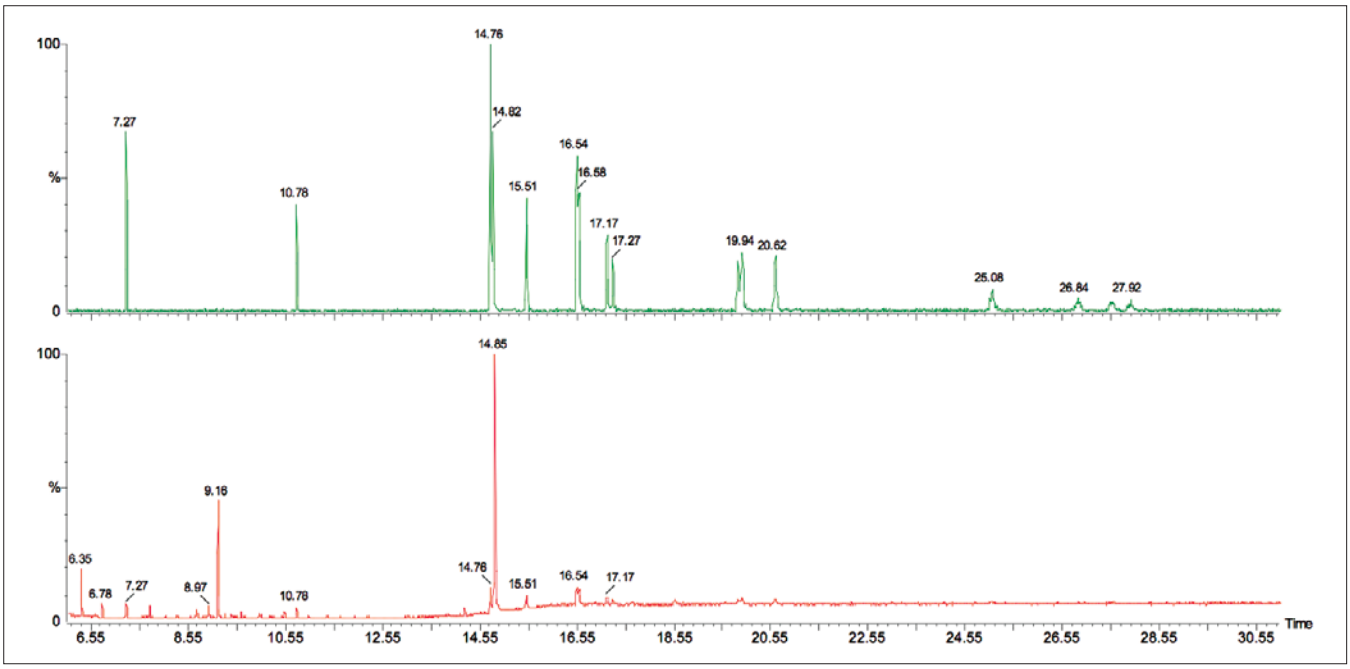


*Figure 1.* Total ion chromatogram (TIC) of 200 ppb PAHs standard solution (lower) and the sum of extracted quantifier ions of PAHs (upper).

*Table 3.* **Calibration Table for PAHs.**

| Name | Retention Time | Quantifier Ion | Qualifier Ion 1 | Qualifier Ion 2 | %RSD of Peak Area (n=7) 100 ng/mL | $r^2$ (5 – 500 ppb) |
|---|---|---|---|---|---|---|
| Naphthalene-D8 | 7.28 | 136 | 137 | 108 | | |
| Phenanthrene-D10 | 10.79 | 188 | 189 | 80 | | |
| Cyclopenta[cd]pyrene | 14.78 | 226 | 227 | 224 | 0.5 | 0.9999 |
| Benz[a]anthracene | 14.78 | 228 | 229 | 226 | 1.1 | 0.9996 |
| Chrysene-D12 | 14.79 | 240 | 241 | 236 | | |
| Chrysene | 14.83 | 228 | 229 | 226 | 3.0 | 0.9999 |
| 5-Methylchrysene | 15.52 | 242 | 241 | 239 | 1.5 | 0.9997 |
| Benzo[b]fluoranthene | 16.56 | 252 | 253 | 250 | 0.3 | 0.9986 |
| Benzo[j]fluoranthene | 16.56 | 252 | 253 | 250 | 0.3 | 0.9986 |
| Benzo[k]fluoranthene | 16.56 | 252 | 253 | 250 | 0.3 | 0.9987 |
| Benzo[a]pyrene | 17.19 | 252 | 253 | 250 | 0.3 | 0.9981 |
| Perylene-D12 | 17.29 | 264 | 265 | 260 | | |
| Indeno[1,2,3-cd]pyrene | 19.89 | 276 | 277 | 138 | 0.4 | 0.9972 |
| Dibenzo(a,h)anthracene | 19.96 | 278 | 279 | 276 | 0.1 | 0.9965 |
| Benzo[ghi]perylene | 20.65 | 276 | 277 | 138 | 0.9 | 0.9997 |
| Dibenzo[a,l]pyrene | 25.09 | 302 | 151 | 150 | 0.3 | 0.9959 |
| Dibenzo[a,e]pyrene | 26.85 | 302 | 151 | 150 | 0.2 | 0.9942 |
| Dibenzo[a,i]pyrene | 27.56 | 302 | 151 | 150 | 0.2 | 0.9913 |
| Dibenzo[a,h]pyrene | 27.94 | 302 | 151 | 150 | 0.2 | 0.9926 |

Following the calibration of the system, 5 g of bacon, preserved ham and sausage were analyzed and the PAH concentrations quantified (Table 4 – Page 5). Table 4 also presents the recoveries of the matrix spike and matrix-spike duplicate, which were fortified with PAHs at 100 ng/mL. The resultant chromatogram for the analysis of the preserved ham sample is pictured in Figure 2.

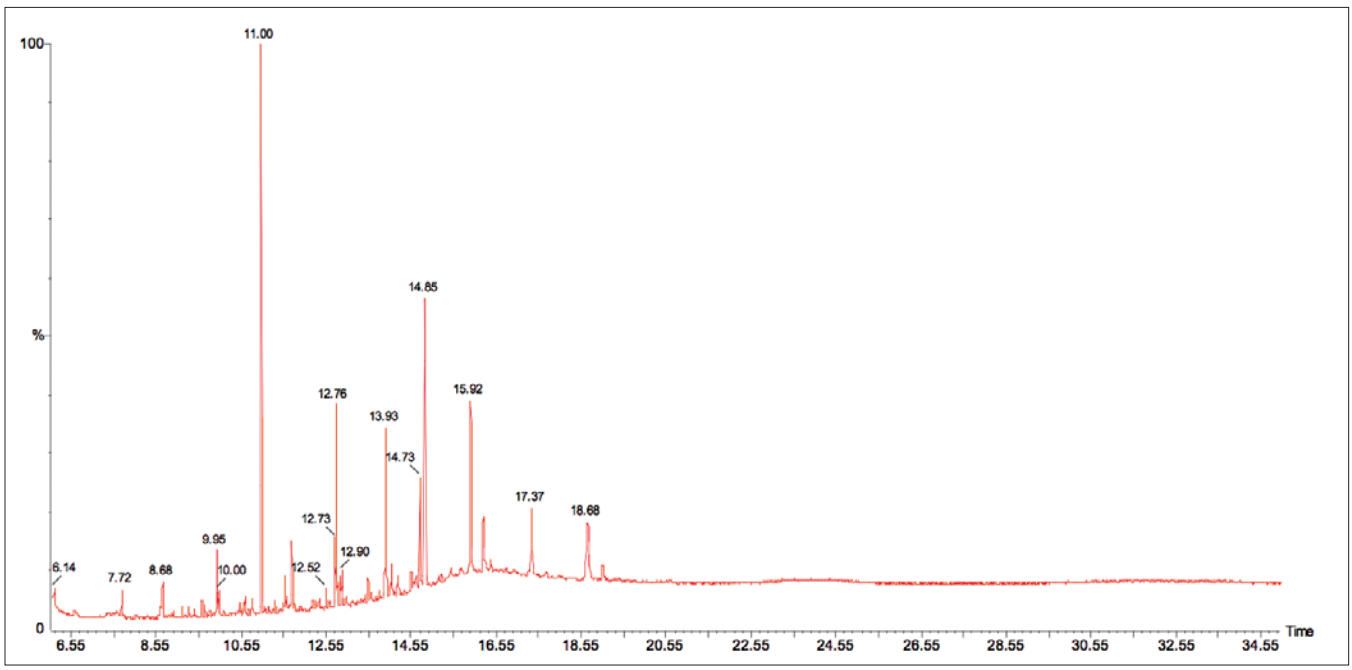


*Figure 2.* Total ion chromatogram (TIC) of the analysis of preserved ham sample.

*Table 4.* **Sample Analysis and Matrix-Spike Recovery.**

| Sample | Compounds | Conc. (ng/mL) | MS Recovery (%) | MSD Recovery (%) |
|---|---|---|---|---|
| Bacon | Cyclopenta[cd]pyrene | 9.3 | 14.6 | 11.6 |
| | Benz[a]anthracene | 22.1 | 78.0 | 76.5 |
| | Chrysene | 18.4 | 76.6 | 75.1 |
| | 5-Methylchrysene | 9.5 | 73.9 | 64.0 |
| | Benzo[b]fluoranthene | 15.2 | 63.4 | 61.7 |
| | Benzo[j]fluoranthene | 15.2 | 63.4 | 61.7 |
| | Benzo[k]fluoranthene | 15.1 | 63.5 | 60.8 |
| | Benzo[a]pyrene | 14.2 | 61.3 | 50.9 |
| | Indeno[1,2,3-cd]pyrene | 11.7 | 47.4 | 36.4 |
| | Dibenzo(a,h)anthracene | 14.3 | 38.0 | 35.1 |
| Preserved Ham | Cyclopenta[cd]pyrene | 12.0 | 11.2 | 13.3 |
| | Benz[a]anthracene | 21.8 | 89.1 | 75.7 |
| | Chrysene | 15.6 | 90.0 | 76.8 |
| | 5-Methylchrysene | 12.0 | 81.6 | 61.9 |
| | Benzo[b]fluoranthene | 17.0 | 73.7 | 63.1 |
| | Benzo[j]fluoranthene | 17.0 | 73.7 | 63.1 |
| | Benzo[k]fluoranthene | 16.9 | 73.8 | 63.3 |
| | Benzo[a]pyrene | 14.4 | 73.1 | 65.5 |
| | Indeno[1,2,3-cd]pyrene | 10.5 | 63.7 | 56.9 |
| | Dibenzo(a,h)anthracene | 12.0 | 40.1 | 44.7 |
| Sausage | Cyclopenta[cd]pyrene | 7.8 | 68.1 | 75.7 |
| | Benz[a]anthracene | 21.6 | 63.3 | 69.1 |
| | Chrysene | 11.3 | 66.3 | 74.4 |
| | 5-Methylchrysene | 6.3 | 70.7 | 78.0 |
| | Benzo[b]fluoranthene | 12.8 | 51.3 | 56.3 |
| | Benzo[j]fluoranthene | 12.8 | 51.3 | 56.3 |
| | Benzo[k]fluoranthene | 12.7 | 51.5 | 53.2 |
| | Benzo[a]pyrene | 12.0 | 50.4 | 54.7 |
| | Indeno[1,2,3-cd]pyrene | 11.6 | 42.2 | 44.7 |
| | Dibenzo(a,h)anthracene | 14.4 | 37.3 | 39.8 |

## Discussion

The matrix-spike recoveries for benzo[ghi]perylene, dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene and dibenzo[a,h]pyrene of all the meat samples was 0%. As was mentioned in the experimental discussion, the focus of this experiment was benzo[a]pyrene, thus the timing of the fraction collection of the GPC was optimized for it. There were high levels of oleic acid in the meat samples. The molecular weight of oleic acid is 282, which is quite close to the molecular weight of benzo [ghi]perylene (MW 276) and dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene, dibenzo [a,h]pyrene(MW 302). When collection time was set from 15 to 20 min, much less matrix interference was observed improving the method performace for benzo[a]pyrene, but eliminating the highest-molecular-weight PAHs.

If the PAHs listed above are of importance, the collection time of the GPC can be changed to 14 to 20 min. The matrix interference will increase, however the recoveries for benzo[ghi]perylene, dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene and dibenzo[a,h]pyrene will improve.

## Conclusion

This application note demonstrates that the Clarus 680 GC/MS system is effective in the analysis of PAHs in meat samples. An effective sample-preparation technique was developed to extract PAHs from meat. GPC was used to remove much of the matrix associated with meat samples. The GC/MS system was calibrated across the range of 5–500 ppb, with a linear response. Three meat samples were analyzed, including samples spiked with known amounts of PAHs, and recoveries reported.

## References


1. DIRECTIVE 2005/69/EC of the European Parliament and of the Council of 16 November 2005.

2. J.A. Gomez-Ruiz, et al., Talanta (2009), doi:10.1016/j.talanta. 2009.07.041.



**PerkinElmer, Inc.**
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
**www.perkinelmer.com**


PerkinElmer®




009139_01