

Gas Chromatography/ Mass Spectrometry

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Determination of 24 Polycyclic Aromatic Hydrocarbons in Smoked Meat by ASE Extraction-GPC Purification Coupled with GC/MS

food.¹ PAHs are formed during the incomplete combustion of organic matter, such as wood utilized in cooking and food preparation. They have attracted wide attention due to studies which have shown the teratogenicity and carcinogenicity of PAH compounds.² Human exposure to PAHs can occur from a number of sources or pathways. Smoking tobacco products is a major contributor to PAH exposure in humans. For non-smokers, food intake is one of the main sources of PAH exposure, and can be the result of contamination from anthropogenic sources, food processing or cooking practices.^{3,4}

The process of smoking meat products, for either preservation or flavor, has been shown to generate PAH contamination. The smoke utilized in the process is known to contain PAH compounds which can migrate into the meats being smoked.⁵ Owing to this known exposure pathway, maximum levels of various PAH compounds have been established to ensure food safety. Examples of these maximum allowable levels in bacon, as stipulated by the European Commission, include 2 µg/kg of benzo(α)pyrene, and a 12 µg/kg total allowable amount of PAH4 (benzo(α)pyrene, benzo(α)anthracene, benzo(b)fluoranthene, and Chrysene).⁶

Introduction

Polycyclic aromatic hydrocarbons (PAHs), a class of complex compounds containing two or more benzene rings, are widely found in the environment and

Currently, the analysis of PAHs in a complex matrix, such as smoked meat, is typically performed with solid phase extraction cartridges or gel permeation chromatography, followed by liquid injection into a HPLC system with fluorescence detector, or a GC/MS system. In this paper, a validated method employing accelerated solvent extraction (ASE) and gel permeation chromatography (GPC), followed by solid phase extraction (SPE) on silica and analytical determination by GC/MS was applied for the detection of PAHs in smoked meat. The results demonstrate that the method is suitable for the simultaneous determination of 24 PAHs in smoked meat with good efficiency, accuracy and reproducibility.

Experimental

Sample Preparation and Extraction

Dionex™ ASE™ Prep DE Diatomaceous Earth was obtained from Thermo Fisher Scientific™. Silica gel 100-200 meshes, for removing polar compounds, was purchased from Qingdao Ocean Chemical Co., Ltd. The chromatographic grade ethyl acetate, cyclohexane and acetonitrile were all obtained from TEDIA® company. Calibration standards (16 EPA-priority PAHs, 15 EU-priority PAHs and Benzo(c)fluorene) and internal standard (16 isotope PAHs) were purchased from Dr. Ehrenstorfer GmbH. The smoked bacon and sausage products were all obtained from local supermarkets and farmers' markets in Chongqing, China. The samples were homogenized and then loaded into a 50 mL centrifuge tube for cryopreservation.

The accelerated solvent extraction procedure used is as follows:

1. Weigh accurately 1.0 g of smoked bacon homogenate, and mix with moderate amounts of diatomaceous earth. Then load them into the extraction cell.
2. Add 50 µl of internal standard, and moderate amounts of solvent ethyl acetate-cyclohexane (v/v=1/1) into the extraction cell.
3. The sample in the extraction cell is heated at 120 °C and 1500 psi for 6 minutes, and then extracted for six minutes. The cycle index is three.
4. The extract is concentrated to 10 mL, and then filtered through a 0.45 µm PFFE membrane.
5. The conditions for GPC system are presented in Table 1. Collect the eluent with eluted times from 8 to 24 minutes in a flask to concentrate to near dryness. Add 1 mL n-hexane into the flask for redissolution.

Table 1. GPC parameters.

| | |
|-------------------------|------------------------------------|
| GPC Column | 25 mm x 400 mm |
| Stationary Phase | Bio-Beads S-X3 |
| Mobile Phase | Ethyl acetate: Cyclohexane (50:50) |
| Flow | 5 mL/min |
| Injection Volume | 5 mL |

The SPE was carried out on a silica gel SPE cartridge. The cleanup procedure is as follows:

1. Weigh 2.5 g deactivated silica gel, and then load into an SPE cartridge.
2. Load the n-hexane solution onto SPE cartridge.
3. Wash the SPE cartridge with 30 mL n-hexane-dichloromethane eluent (v/v=8/2).
4. Concentrate eluent to near dryness.

The dry sample is then reconstituted in an autosampler vial with 0.5 mL of acetonitrile for GC/MS analysis.

The precision and recovery were investigated by spiking three fresh meat samples with 10 µL, 50 µL and 100 µL of 1 µg/mL 24 PAHs standard solution. Method detection limits were determined by analyzing standards of 1.0 and 5.0 µg/L to determine which concentration gave a signal-to-noise ratio of 3.

Instrumentation

A PerkinElmer Clarus® SQ8 GC/MS was utilized in these experiments, with the conditions presented in Table 2. An Agilent J&W DB-EUPAH column (20 m x 0.18 mm x 0.14 µm) was used to separate the eluting compounds. The Clarus SQ8 offers an ideal GC/MS solution for the determination of a variety of volatile and semi-volatile compounds, providing good sensitivity and stability.

Table 2. GPC parameters.

| GC Parameters | | | |
|---------------------------------|--|--------------|--------------|
| Injector Type | Programmable split/splitless injector with capillary split/splitless liner with wool | | |
| Inlet Temp | 250 °C | | |
| Injection Volume | 1.5 µL | | |
| Carrier Gas | Helium | | |
| Carrier Gas Flow Rate | 0.7 mL/min | | |
| Initial Oven Temp | 80 °C | | |
| Oven Hold | 2 min | | |
| Ramp | 10 °C/min | | |
| 2nd Oven Temp | 250 °C | | |
| Oven Hold | 2 min | | |
| Ramp | 8 °C/min | | |
| 3rd Oven Temp | 315 °C | | |
| Oven Hold | 18 min | | |
| Instrument Time Event | Time | Event | Value |
| | -0.6 min | Car | 4 mL/min |
| | -0.5 min | Spl | Off |
| | 1.0 min | Spl | 20 mL/min |
| | 1.2 min | Car | 0.7 mL/min |
| MS Parameters | | | |
| GC Inlet Line Temp | 290 °C | | |
| Ion Source Temp | 240 °C | | |
| Solvent Delay | 5 min | | |
| Function Type | SIFI | | |

Calibration

Calibration details are as follows:

- Benzo(c)fluorene stock standard solution (200 µg/mL): Weigh 10 mg Benzo(α)fluorene. Dissolve it in acetonitrile and dilute to volume in a 50 mL volumetric flask.
- 24 PAHs standard solution: Dilute 10 µL of 16 EPA-priority PAHs stock solution, 10 µL of Benzo(α)fluorene stock solution and 100 µL of 15 EU-priority PAHs to 2 mL by acetonitrile.
- Internal standard (IS) solution: Dilute 16 isotope PAHs stock solution 50 times by acetonitrile. The resulting solution contains each standard at a concentration of 2 µg/mL.

The calibration curve was prepared by dissolving the 24 PAHs standard solution and internal standard solution in 1 mL of acetonitrile, resulting in a concentration of 100 µg/L of each internal standard.

Results and Discussion

The total ion chromatogram of a 1 µg/mL standard (Figure 1) shows baseline resolution of the target compounds. Table 3 shows the qualitative and quantitative ions of 24 PAHs and 16 isotope internal standards. The calibration curves were plotted as the peak area ratios between the quantification ions for the analytes and the respective IS, versus the amounts of analytes. The determination coefficient (r^2) was over 0.997, showing the reliability of the analysis in the calibration range (Table 4).

Table 5 summarizes the results for the method detection limits (MDLs), percent recoveries and repeatability. By the approach mentioned above, the MDLs per sample were calculated to be in the range of 0.4–5.0 µg/kg for PAHs; the recoveries are in the range of 70.4–118.5%; the precision data (RSD%) are in the range of 5.43–9.74%. The results for precision, linearity, recovery and method detection limit are excellent for all compounds.

Table 3. The qualitative and quantitative ions of 24 PAHs and 16 isotope internal standards.

| NO. | RT | Compound Name | Quantitation Ion | Qualitative Ion | Internal Standard | Quantitation Ion for IS |
|-----|-------|------------------------|------------------|-----------------|----------------------------|-------------------------|
| 1 | 8.02 | naphthalene | 128 | 102,129 | naphthalene-d8 | 136 |
| 2 | 12.16 | acenaphthylene | 152 | 151,76 | acenaphthylene-d8 | 160 |
| 3 | 12.48 | acenaphthene | 154 | 152,153 | acenaphthene-d10 | 164 |
| 4 | 13.70 | fluorene | 166 | 165,83 | fluorene-d10 | 176 |
| 5 | 16.36 | phenanthrene | 178 | 176,152 | Phenanthrene-d10 | 188 |
| 6 | 16.44 | anthracene | 178 | 176,152 | anthracene-d10 | 188 |
| 7 | 19.43 | fluoranthene | 202 | 200,203 | fluoranthene-d8 | 208 |
| 8 | 20.25 | pyrene | 202 | 101,200 | pyrene-d8 | 208 |
| 9 | 21.45 | benzo(c)fluorene | 216 | 213,215 | benz(a)anthracene-d12 | 240 |
| 10 | 24.32 | benz(a)anthracene | 228 | 226,229 | benz(a)anthracene-d12 | 240 |
| 11 | 24.56 | cyclopenta(c,d)pyrene | 226 | 224,227 | chrysene-d12 | 240 |
| 12 | 24.61 | chrysene | 228 | 226,229 | chrysene-d12 | 240 |
| 13 | 26.00 | 5-methyl chrysene | 242 | 239,241 | chrysene-d12 | 240 |
| 14 | 27.79 | benz(b)anthracene | 252 | 250,253 | benz(b)anthracene-d12 | 264 |
| 15 | 27.86 | benz(k)anthracene | 252 | 250,253 | benz(b)anthracene-d12 | 264 |
| 16 | 27.96 | benz(j)anthracene | 252 | 250,253 | benz(b)anthracene-d12 | 264 |
| 17 | 28.96 | benzo(a)pyrene | 252 | 250,253 | benzo(a)pyrene-d12 | 264 |
| 18 | 32.28 | indeno(1,2,3-cd)pyrene | 276 | 274,277 | indeno(1,2,3-cd)pyrene-d12 | 284 |
| 19 | 32.29 | dibenz(a,h)anthracene | 278 | 276,279 | dibenz(a,h)anthracene-d14 | 292 |
| 20 | 33.53 | benzo(g,h,i)perylene | 276 | 274,277 | benzo(g,h,i)perylene-d12 | 288 |
| 21 | 39.27 | dibenzo(a,l)pyrene | 302 | 150,300 | benzo(g,h,i)perylene-d12 | 288 |
| 22 | 41.43 | dibenzo(a,e)pyrene | 302 | 150,300 | benzo(g,h,i)perylene-d12 | 288 |
| 23 | 42.82 | dibenzo(a,i)pyrene | 302 | 150,300 | benzo(g,h,i)perylene-d12 | 288 |
| 24 | 43.63 | dibenzo(a,h)pyrene | 302 | 150,300 | benzo(g,h,i)perylene-d12 | 288 |

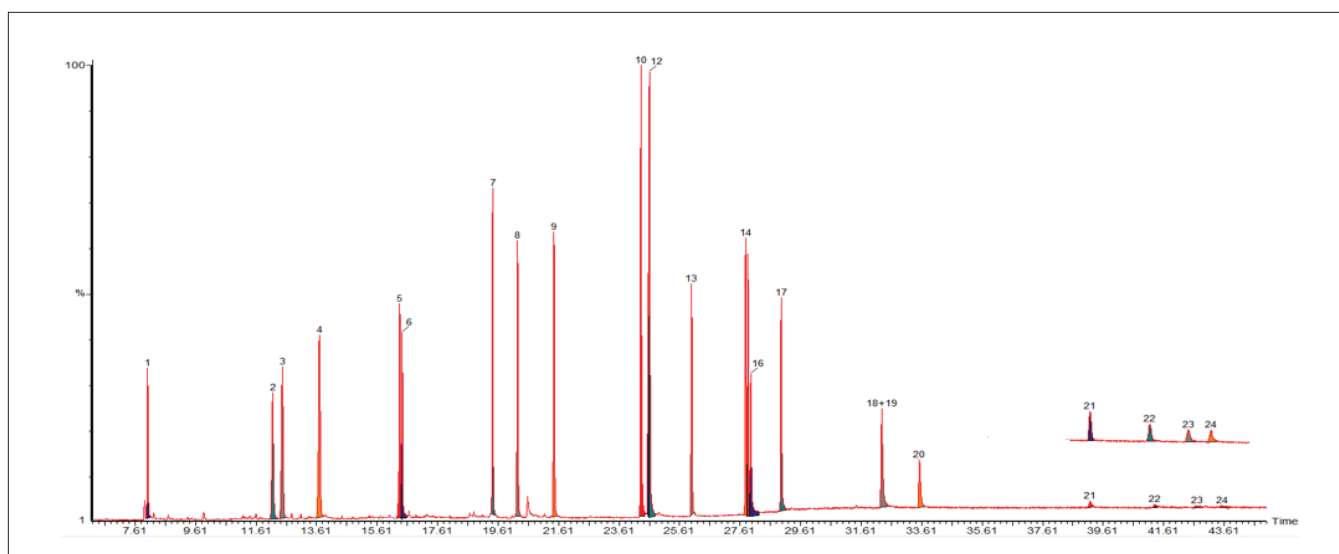


Figure 1. The total ion chromatogram of a 1 µg/mL 24 PAHs standard.

Table 4. Results for linearity.

| Compound Name | Curve Range µg/L | r ² | Calibration Curve |
|------------------------|------------------|----------------|-------------------|
| naphthalene | 1-300 | 0.9997 | Y=0.76x-1.87 |
| acenaphthylene | 1-300 | 0.9998 | Y=0.85x-0.82 |
| acenaphthene | 1-300 | 0.9999 | Y=0.90x-1.09 |
| fluorene | 1-300 | 0.9998 | Y=0.80x-1.26 |
| phenanthrene | 1-300 | 0.9998 | Y=0.83x-0.20 |
| anthracene | 1-300 | 0.9996 | Y=0.70x-0.30 |
| fluoranthene | 1-300 | 0.9998 | Y=5.03x-10.28 |
| pyrene | 1-300 | 0.9997 | Y=4.20x+1.73 |
| benzo(c)fluorene | 1-300 | 0.9997 | Y=0.86x-2.30 |
| benz(a)anthracene | 2-600 | 0.9990 | Y=2.01x-9.28 |
| cyclopenta(c,d)pyrene | 1-300 | 0.9995 | Y=1.39x-4.75 |
| chrysene | 2-600 | 0.9996 | Y=1.81x-5.95 |
| 5-methyl chrysene | 1-300 | 0.9998 | Y=0.55x-1.21 |
| benz(b)anthracene | 2-600 | 0.9993 | Y=2.00x-7.64 |
| benz(k)anthracene | 2-600 | 0.9993 | Y=2.09x-8.27 |
| benz(j)anthracene | 1-300 | 0.9992 | Y=0.83x-3.59 |
| benzo(a)pyrene | 2-600 | 0.9997 | Y=1.94x-4.27 |
| indeno(1,2,3-cd)pyrene | 2-600 | 0.9996 | Y=8.41x-27.02 |
| dibenz(a,h)anthracene | 2-600 | 0.9976 | Y=1.52x-9.26 |
| benzo(g,h,i)perylene | 2-600 | 0.9993 | Y=1.57x-1.90 |
| dibenzo(a,l)pyrene | 5-300 | 0.9997 | Y=0.31x+0.026 |
| dibenzo(a,e)pyrene | 5-300 | 0.9989 | Y=0.25x-0.93 |
| dibenzo(a,i)pyrene | 5-300 | 0.9975 | Y=0.17x-1.14 |
| dibenzo(a,h)pyrene | 5-300 | 0.9976 | Y=0.15x-1.03 |

Measurements of PAHs in Actual Sample

100 smoked meat samples were analyzed in order to investigate the practicability of this method (Table 6). According to the EU standard, the detection rate for benzo(a)pyrene is 76%, and

100% for the total amount of PAH4; the average rate of exceeding the allowable limit is 76% for benzo(a)pyrene and 33% for the total amount of PAH4.

Table 5. Results for MDL, repeatability and recovery.

| Compound Name | Spiking Volume | | | | | | MDL (µg/kg) |
|------------------------|----------------|-------|------------|-------|------------|-------|-------------|
| | 10 µL | | 50 µL | | 100 µL | | |
| | Recovery % | RSD % | Recovery % | RSD % | Recovery % | RSD % | |
| naphthalene | 85.2 | 7.81 | 90.3 | 6.78 | 94.2 | 5.45 | 0.5 |
| acenaphthylene | 87.5 | 9.74 | 94.5 | 7.65 | 102.2 | 6.54 | 0.4 |
| acenaphthene | 81.4 | 8.64 | 98.6 | 6.67 | 101.5 | 5.76 | 0.9 |
| fluorene | 83.3 | 7.65 | 90.4 | 7.78 | 100.5 | 6.54 | 0.6 |
| phenanthrene | 85.9 | 8.61 | 78.3 | 7.86 | 92.4 | 6.76 | 0.4 |
| anthracene | 89.4 | 7.85 | 101.1 | 7.56 | 105.2 | 5.87 | 0.7 |
| fluoranthene | 85.7 | 6.94 | 88.3 | 7.59 | 94.5 | 6.76 | 0.5 |
| pyrene | 88.6 | 7.84 | 106.2 | 7.79 | 105.5 | 7.69 | 0.5 |
| benzo(c)fluorene | 89.4 | 8.64 | 90.6 | 7.65 | 98.4 | 7.65 | 0.6 |
| benz(a)anthracene | 88.4 | 7.85 | 80.5 | 6.78 | 88.3 | 6.89 | 0.6 |
| cyclopenta(c,d)pyrene | 70.6 | 7.79 | 70.4 | 6.97 | 71.1 | 6.83 | 0.5 |
| chrysene | 75.3 | 8.76 | 80.7 | 7.89 | 88.3 | 6.53 | 0.5 |
| 5-methyl chrysene | 75.7 | 8.74 | 82.6 | 7.67 | 88.1 | 6.45 | 0.4 |
| benz(b)anthracene | 78.3 | 8.64 | 80.7 | 6.89 | 86.4 | 6.54 | 0.4 |
| benz(k)anthracene | 80.9 | 8.96 | 82.8 | 7.89 | 90.8 | 6.78 | 0.7 |
| benz(j)anthracene | 80.2 | 8.75 | 80.6 | 7.87 | 91.8 | 7.89 | 1.0 |
| benzo(a)pyrene | 87.1 | 8.91 | 88.9 | 7.65 | 90.6 | 6.98 | 0.5 |
| indeno(1,2,3-cd)pyrene | 78.3 | 8.65 | 84.4 | 7.85 | 80.4 | 6.89 | 0.9 |
| dibenz(a,h)anthracene | 85.8 | 7.9 | 90.3 | 8.73 | 85.6 | 6.65 | 3.0 |
| benzo(g,h,i)perylene | 85.3 | 7.86 | 88.5 | 8.67 | 92.3 | 6.89 | 1.2 |
| dibenzo(a,l)pyrene | 75.7 | 8.75 | 104.3 | 7.75 | 91.4 | 6.71 | 5.0 |
| dibenzo(a,e)pyrene | 77.8 | 7.9 | 112.1 | 7.81 | 90.4 | 6.21 | 5.0 |
| dibenzo(a,i)pyrene | 79.8 | 8.9 | 80.4 | 5.89 | 85.2 | 5.43 | 5.0 |
| dibenzo(a,h)pyrene | 87.9 | 9.06 | 118.5 | 6.82 | 97.7 | 5.67 | 5.0 |

Table 6. The results of 24 PAHs in 100 smoked meat samples.

| Compound Name | Minimum µg/kg | Maximum µg/kg | Detection Rate % | Detection Rate % |
|------------------------|---------------|---------------|------------------|------------------|
| naphthalene | 25.0 | 1489.8 | 333.9 | 100 |
| acenaphthylene | 14.6 | 1496.4 | 386.7 | 90 |
| acenaphthene | 2.4 | 2590.6 | 270.0 | 90 |
| fluorene | 98.2 | 913.6 | 278.1 | 85 |
| phenanthrene | 76.7 | 4927.1 | 983.7 | 100 |
| anthracene | 17.9 | 748.4 | 214.6 | 95 |
| fluoranthene | 14.9 | 524.8 | 104.3 | 95 |
| pyrene | 14.3 | 612.3 | 121.9 | 95 |
| benzo(c)fluorene | 7.9 | 77.9 | 24.4 | 71 |
| benz(a)anthracene | 2.6 | 11.4 | 4.2 | 100 |
| cyclopenta(c,d)pyrene | 0.7 | 24.6 | 6.1 | 100 |
| chrysene | 0.5 | 17.4 | 3.5 | 100 |
| 5-methyl chrysene | 3.8 | 4.4 | 4.1 | 19 |
| benz(b)anthracene | ND | 2.0 | 0.6 | 38 |
| benz(k)anthracene | 2.6 | 8.4 | 4.1 | 67 |
| benz(j)anthracene | 6.2 | 9.5 | 7.6 | 38 |
| benzo(a)pyrene | ND | 3.6 | 2.4 | 76 |
| indeno(1,2,3-cd)pyrene | 2.7 | 3.2 | 3.0 | 24 |
| dibenz(a,h)anthracene | ND | ND | ND | 0 |
| benzo(g,h,i)perylene | ND | ND | ND | 0 |
| dibenzo(a,l)pyrene | ND | ND | ND | 0 |
| dibenzo(a,e)pyrene | ND | ND | ND | 0 |
| dibenzo(a,i)pyrene | ND | ND | ND | 0 |
| dibenzo(a,h)pyrene | ND | ND | ND | 0 |
| Total amount of PAH4 | 3.2 | 34.4 | 10.1 | 100 |

Summary

In this study, the method of determination for 24 PAHs in smoked meats was established by ASE extraction-GPC purification coupled with GC/MS. The precision, recovery and linearity achieved by the method ensure a reliable determination of PAHs at ultra-trace levels, which demonstrates the compliance with regulatory requirements.

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