



APPLICATION NOTE

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

Introduction

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

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

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		
2 nd Oven Temp	250 °C		
Oven Hold	2 min		
Ramp	8 °C/min		
3 rd 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

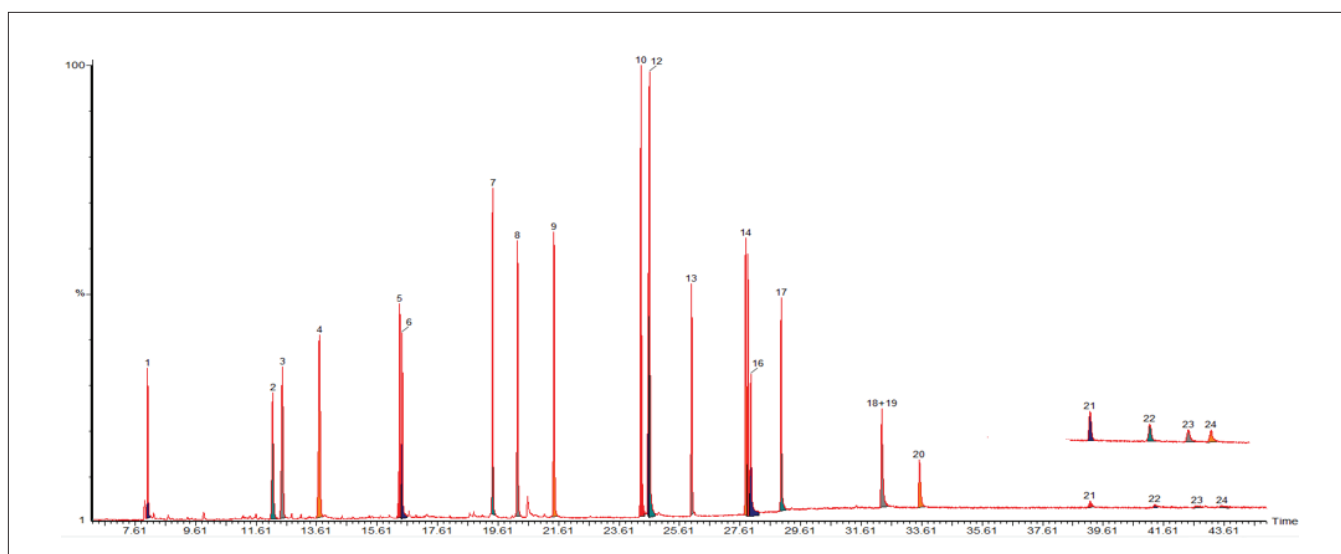


Figure 1. The total ion chromatogram of a 1 µg/mL 24 PAHs standard.

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.

References

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