# Evaluation of Analysis of Polycyclic Aromatic Hydrocarbons in Meat Products by Liquid Chromatography

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Several extraction, separation, and detection methods of polycyclic aromatic hydrocarbons (PAHs) in meat products were evaluated by using liquid chromatography. Results showed that Soxhlet extraction of PAHs followed by purification with a Sep-Pak Florisil cartridge removed more impurities than the sonication method. With HPLC, a mobile phase of acetonitrile−water (55:45, v:v) was maintained for 2 min, linearly programmed to 100% acetonitrile over a 23 min period, and maintained for 15 min. Sixteen PAHs were separated by a $C_{18}$ column and detected by UV at 254 nm, while 15 PAHs were detected with fluorescence. The latter method was found to have 20−320 times higher sensitivity than the former. The following settings (excitation wavelength/emission wavelength) were used for fluorescence: $\lambda_1 = 270$ nm/340 nm (naphthalene, acenaphthene, fluorene); $\lambda_2 = 254$ nm/375 nm (phenanthrene); $\lambda_3 = 260$ nm/420 nm (anthracene, fluoranthene); $\lambda_4 = 254$ nm/390 nm (pyrene, benzo[*a*]anthracene, chrysene), $\lambda_5 = 260$ nm/420 nm (benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene); $\lambda_6 = 293$ nm/498 nm (indeno[1,2,3-*c*,*d*]pyrene). The presence of PAHs in some commercial meat products was also determined.

**Keywords:** *Polycyclic aromatic hydrocarbon; meat products; liquid chromatography*

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), formed through incomplete combustion or pyrolysis of wood gasoline, can be detrimental to human health if consumed in significant amount. To date more than 100 PAHs have been characterized in nature, 16 of which were classified as "priority pollutants" according to the U.S. Environmental Protection Agency (EPA) (Wise et al., 1993). Of these 16 PAHs, benzo[*a*]pyrene and dibenz[*a*,*h*]anthracene were reported to be the most carcinogenic (IARC, 1983, 1987). In 1987 the joint FAO/WHO Expert Committee on Food Additives declared that the amount of benzo[*a*]pyrene in foods should not exceed 10 ppb. As environmental pollution in Taiwan is becoming a serious problem, it is possible that PAHs may be widely distributed in the environment and thus contaminate foods. In addition, some PAHs can be formed during the smoking and grilling of foods (Rhee and Bratzler, 1970; Lo and Sandi, 1978; Kramers and Van Der Heljden, 1988; Gomma et al., 1993; Yakibu et al., 1993). Since many PAHs have been proved to be cytotoxic and carcinogenic (Zedeck, 1980; IARC, 1983, 1987), it is necessary to develop a fast and accurate method for the determination of PAHs in foods.

Due to the presence of trace amounts of PAHs in foods, the determination of PAHs has been difficult. The major problems associated with the analysis of PAHs in foods are as follows: (1) most PAHs are present in ppb or ppt in foods, which make their extraction difficult; (2) many organic compounds can be coextracted with PAHs from foods, which can interfere with the subsequent separation and identification of PAHs; and (3) most PAHs are structurally similar and present in isomeric forms, which makes their separation and identification difficult. The most common method for the isolation of PAHs from foods usually involves saponification of lipids by methanolic KOH, followed by liquid−liquid partition and liquid−solid chromatography. Joe et al. (1982) extracted PAHs from wheat germ by sonication with cyclohexane as the extracting solvent, followed by purification with partition and column chromatography. The recovery of PAHs obtained was between 78 and 97%. Kolarovic and Traitler (1982) extracted PAHs from vegetable oil by cyclohexane and a caffeine−formic acid solution, followed by purification with a silica gel column and high-performance thin-layer chromatography (HPTLC). In another study, Joe et al. (1984) used 1,1,2-trichloro-1,2,2-trifluoroethane to extract PAHs from alcoholic KOH digests of smoked meat, followed by purification with a deactivated silica gel−alumina column and partition between dimethyl sulfoxide and cyclohexane. Lawrence and Weber (1984a) extracted PAHs from meat and fish products by saponification of lipids with methanolic KOH under reflux, followed by purification with a Florisil column and partition with dimethyl sulfoxide and hexane. Takatsuki et al. (1985) extracted PAHs from fish by alkaline digestion, extraction with *n*-hexane, and purification with a silica gel column. A similar study was conducted by Hopia et al. (1986), who used cyclohexane and dimethylformamide to extract PAHs from vegetable oil, followed by purification with a $SiO_2$ column. Coates and Elzerman (1986) pointed out that the extraction of PAHs from plant tissue by sonication is a convenient and reliable method. Karlesky et al. (1986) compared two extraction procedures, i.e., dimethyl sulfoxide−pentane partition and solid phase (amino-bonded packing material) extraction, and found that the former resulted in lower recovery than the latter. Dong et al. (1993) developed a fast method to extract PAHs from soil, water, and waste oil. This method is easy because only sonication was conducted for extraction; however, the HPLC system might be contaminated. In meat products, many components such as protein, lipid, and PAH-

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like substances have to be removed prior to the separation of PAHs. As many authors have used alkaline digestion, sonication, column chromatography, and liquid−liquid partition for the isolation of PAHs from food samples, it is necessary to compare the extraction efficiency of these methods when applied to meat products.

The separation of PAHs previously has been achieved by thin-layer chromatography (TLC). Doremire et al. (1979) used TLC to determine 3,4-benzopyrene in roasted meat. However, this method is lengthy and failed to resolve various PAHs in foods. Gas chromatography (GC) has been widely used to determine PAHs in foods (Lee and Novotny, 1976; Kolarovic and Traitler, 1982; Afolabi et al., 1983; Hopia et al., 1986; Tuominen et al., 1988). Although capillary GC has high resolution power, PAHs might be degraded because of exposure to high temperature during separation. Also, a number of isomeric PAHs such as benzo[*b*]fluoranthene and benzo[*k*]fluoranthene are difficult to separate (Wise et al., 1993). To remedy this problem, many high-performance liquid chromatographic methods were developed to separate PAHs from foods. Schmit et al. (1971) were the first to use a chemically bonded $C_{18}$ column to separate PAHs. Since then, reversed-phase HPLC based on a $C_{18}$ column has become the most popular LC mode for separation of PAHs (Wise et al., 1977; Ogan et al., 1979; Joe et al., 1984; Takatsuki et al., 1985; Sander and Wise, 1989, 1990; Dong et al., 1993; Wise et al., 1993; Gomma et al., 1993; Yakibu et al., 1993). Although the separation power of HPLC is theoretically inferior to that of GC, HPLC can still provide an ideal means for the fractionation of PAH eluates for subsequent analysis by spectroscopic techniques.

Of the various HPLC methods, fluorescence detection has been found to be very suitable for the quantification of PAHs in a variety of foods because of its high sensitivity. Nevertheless, in many published reports the number of PAHs detected by fluorescence is still limited. Therefore, it is necessary to develop a better wavelength-programming method and to compare the difference in detection of 16 PAHs by UV and fluorescence.

The presence of PAHs in foods has been investigated by many researchers. Lawrence and Weber (1984b) determined PAHs in Canadian samples of processed vegetable and dairy products by HPLC and found that the use of direct heating for drying milk powders and malt could lead to elevated levels of PAHs. Joe et al. (1984) developed a liquid chromatographic method for the determination of PAHs in some smoked products and found that carcinogenic and noncarcinogenic PAHs were present in trace levels (<1 ppb). In another study, Lawrence and Weber (1984a) determined 18 PAHs in some Canadian commercial fish, shellfish, and meat products by HPLC with confirmation by capillary GC−MS. Most samples were found to contain carcinogenic PAHs in the low-to-submicrograms per kilogram range. A similar study was conducted by Takatsuki et al. (1985), who quantified PAHs in fish and shellfish by HPLC and found that a number of PAHs, such as benzo[*a*]pyrene, were decomposed when exposed to light, oxygen, and alkaline conditions. Also, five PAHs ranging from 0 to 2.64 ppb were found. The presence of benzo[*a*]pyrene in liquid smoke flavoring and smoked foods was investigated by Yabiku et al. (1993), who reported that benzo[*a*]pyrene concentrations varied from 0.1 to 36.6 μg/kg for the former and from 0.1 to 5.9 μg/kg for the latter. Gomma et al. (1993) further reported that the total PAH concentrations in meat products ranged from 2.6 μg/kg in a cooked ham sample to 29.8 μg/kg in grilled pork chops, while those in fish products ranged from 9.3 μg/kg in smoked shrimp to 86.6 μg/kg in smoked salmon. As some traditional Chinese meat products such as grilled duck, grilled chicken, stewed chicken heart, and chicken wing are quite popular in Taiwan, the possibility of the presence of PAHs in these products also has to be investigated.

The purposes of this study were to evaluate several extraction, separation, and detection methods for the determination of PAHs in grilled duck and to apply the most appropriate method for the quantification of PAHs in several meat commodities in Taiwan.

## MATERIALS AND METHODS

**Materials.** Sixteen PAH standards were purchased from Supelco Co. (Bellefonte, PA), and each was dissolved in $CH_2Cl_2$−$CH_3OH$ (1:1, v:v) for the following concentrations: acenaphthene, 1000 ppm; fluorene, 200 ppm; phenanthrene, 100 ppm; anthracene, 1000 ppm; fluoranthene, 200 ppm; pyrene, 100 ppm; benzo[*a*]anthracene, 100 ppm; chrysene, 100 ppm; benzo[*b*]fluoranthene, 200 ppm; benzo[*k*]fluoranthene, 100 ppm; benzo[*a*]pyrene, 100 ppm; dibenz[*a*,*h*]anthracene, 200 ppm; benzo[*g*,*h*,*i*]perylene, 100 ppm; indeno[1,2,3-*c*,*d*]pyrene, 100 ppm.

Solvents used for the extraction of PAHs, including methanol, *n*-hexane, methylene chloride, cyclohexane, and dimethyl sulfoxide (DMSO), were from Merck Co. (Darmstadt, Germany). The HPLC-grade solvents, such as methanol and acetonitrile, were from Mallinckrodt Co. (Paris, KY) and were degassed under vacuum and filtered through a 0.2 μm membrane filter prior to use. Deionized water was obtained from a Milli-Q water purification system by Millipore Co. (Bedford, MA).

Twenty meat samples, including stewed chicken breast, stewed chicken wing, stewed chicken liver, stewed chicken heart, grilled chicken, grilled duck, stewed pork, stewed pork stomach, smoked Frankfurt sausage, and smoked pork, were purchased from a local market in Taipei.

**Instrumentation.** The HPLC instrument consisted of a Shimadzu SPD-M6A photodiode array detector (Kyoto, Japan), a Jasco 970/975 UV/vis detector, a Jasco 821-FP fluorescence detector, two Jasco PU-980 pumps (Tokyo, Japan), and an SIC Chromatocoder 12 integrator (System Instruments Co., Tokyo, Japan). A Phenomenex stainless steel ODE-3029-ED Envirosep-pp $C_{18}$ column (125 × 4.6 mm i.d.) containing 5 μm particle size was used. The Sep-Pak Florisil cartridge containing 960 mg of packing material was from Waters Co. (Milford, MA).

**Extraction of PAHs in Grilled Duck by the Sonication Method.** The extraction and purification methods were similar to those described by Lawrence and Weber (1984a,b) Joe et al. (1984), Natusch and Tomkins (1978), and Dong et al. (1993). Five grams of duck meat was freeze-dried for 48 h before it was ground into a fine powder with a grinder. The sample was placed in a 100 mL volumetric flask and extracted with 20 mL of methylene chloride or hexane. The mixture was sonicated for 30 min and filtered through anhydrous sodium sulfate. Then the extract was transferred to a 50 mL flask and concentrated to 1 mL at 35 °C by using a rotary evaporator. A 1 mL extract was poured into a Sep-Pak Florisil cartridge, which was previously activated with 10 mL of methylene chloride and 20 mL of hexane with a flow rate of 2.0 mL/min. Two elution solvent systems, A and B, were used to elute PAHs: A consists of 10 mL of hexane followed by 8 mL of hexane−methylene chloride (3:1, v:v), while B consists of 18 mL of hexane−methylene chloride (3:1, v:v). The eluate was collected for either system, evaporated to dryness, dissolved in 1 mL of methanol−methylene chloride (1:1, v:v), and filtered through a 0.2 μm membrane filter prior to HPLC analysis.

**Extraction of PAHs in Grilled Duck by the Soxhlet Method.** The extraction and purification methods were similar to those described by Joe et al. (1984) and Takatsuki et al. (1985). Thirty grams of duck meat was cut into pieces and freeze-dried before placement in a round filter paper. The paper was centered in a Soxhlet extractor for the extraction of fat from the sample. A 500 mL round bottomed flask, to which methanol (200 mL) and 50% potassium hydroxide (25 mL) were added, was connected to the bottom of the Soxhlet extractor. After reflux for 3 h, the alkaline mixture was cooled to 40 °C, and by portions 150 mL of *n*-hexane was added with occasional swirling. Then the solution was poured into a 500 mL separatory funnel containing 150 mL of water. The flask was rinsed with 10 mL of methanol twice and the rinses were added to the separatory funnel, which was then skaken vigorously and set aside to separate into aqueous and organic layers. The former was extracted twice with 150 and 100 mL of *n*-hexane. Then the *n*-hexane extracts were all combined, washed with 100 mL of water three times, and dried over anhydrous sodium sulfate. The dried *n*-hexane extract was poured into a 500 mL flask and concentrated to 1 mL by a rotary evaporator. The 1 mL concentrate was poured into a Sep-Pak Florisil cartridge, which had been previously activated with 10 mL of methylene chloride and 20 mL of hexane with a flow rate of 2.0 mL/min. Elution solvents consisting of 10 mL of *n*-hexane followed by 8 mL of *n*-hexane−methylene chloride (3:1, v:v) were passed through the cartridge. The eluate was collected, evaporated to dryness, and dissolved in 1 mL of methanol−methylene chloride (1:1, v:v). The solution was filtered through a 0.2 $\mu$m membrane filter and stored in a vial filled with nitrogen gas for HPLC analysis.

To determine whether the eluate from a Sep-Pak Florisil cartridge needs further purification, a method similar to that described by Joe et al. (1984) was used. The eluate was transferred to a flask for rotary evaporation and 10 mL of DMSO was added. After evaporation of methanol and methylene chloride, the DMSO concentrate was poured into a 125 mL separatory funnel. The flask was washed with 50 mL of cyclohexane and added to the funnel. The funnel was shaken vigorously and set aside to form two layers. The bottom layer (DMSO layer) was poured into a 250 mL separatory funnel containing 25 mL of cyclohexane and 90 mL of water. The upper layer (cyclohexane layer) was extracted twice with 15 mL of DMSO and the cyclohexane layer was discarded. The DMSO layers were added to the 250 mL separatory funnel, and the funnel was shaken vigorously for 2 min to form two layers. The bottom layer was transferred to a second 250 mL separatory funnel containing 25 mL of cyclohexane; the funnel was shaken for 2 min and two layers formed. The lower layer (aqueous layer) was discarded, and the two cyclohexane extracts (upper layer) were combined into the first 250 mL funnel. The second 250 mL funnel was rinsed with two 10 mL portions of cyclohexane, and the rinses were added to the first 250 mL funnel. The solution was washed three times with 100 mL of water, and the aqueous layer was discarded. The cyclohexane extract was dried over anhydrous sodium sulfate, evaporated to dryness, and dissolved in 1 mL of methanol−methylene chloride (1:1, v:v). The solution was filtered through a 0.2 $\mu$m membrane filter and transferred to a vial filled with nitrogen gas for HPLC analysis.

**HPLC Analysis of PAHs.** One isocratic solvent system and two gradient systems were used to compare the separation efficiency of 16 PAHs. The former consisted of acetonitrile−water (70:30, v:v) with a flow rate of 2.0 mL/min. The latter consisted of a mixture of acetonitrile−water with a flow rate of 1.25 mL/min. One started with 40% acetonitrile for 2 min, was then linearly programmed to 100% acetonitrile within 25 min, and was maintained for 13 min, while the other started with 55% acetonitrile for 2 min, was then linearly programmed to 100% acetonitrile within 23 min, and was maintained for 15 min. A mixture of 16 PAH standards was diluted 100 times by acetonitrile and injected onto the HPLC. The injection volume was 20 $\mu$L, with UV detection at 254 nm and sensitivity at 0.08 AUFS. The capacity factor ($k'$) was used to determine the separation efficiency of the 16 PAHs. In addition to 254 nm, the other six wavelengths, 230, 245, 270, 295, 335, and 360 nm, were also compared with respect to the peak area responses of the 16 PAHs. For fluorescence detection, the following settings (excitation wavelength/emission wavelength) were compared: 240 nm/370 nm, 270 nm/340 nm, 260 nm/420 nm, 254 nm/375 nm, 293 nm/498 nm, and 254 nm/390 nm. The detection limit was calculated as the minimum sample size that produced a signal 3 times the peak height to noise ratio. PAHs were quantified by using an external calibration method. Four concentrations of each PAH ranging from 0.5 ppb to 20 ppm, were injected onto the HPLC, and the calibration curve for each PAH standard was obtained by plotting concentration against area. The regression equation and correlation coefficient ($r^2$) were calculated using CHEN-WIN computer software system (Shuen-Hua Co., Taipei, Taiwan). The recovery was obtained by adding a 100 $\mu$L mixture of the 16 PAHs to a duck sample, and extraction was performed by sonication and Soxhlet methods as described earlier. After quantification by HPLC, the recovery data for both methods were obtained by dividing the amount of PAHs following extraction and HPLC analysis by the amount of PAHs added to the sample. The recovery data were also subjected to analysis of variance and Duncan's multiple range test. The PAHs present in meat samples were identified by comparison of the retention times and spectra of unknown peaks with those of reference standards and by the addition of PAH standards to the sample for cochromatography. Each PAH in the sample was quantified by using the formula

$$W_s = (A_s - b)/a/R$$

where $W_s$ is the PAH concentration in the sample, $A_s$ is the peak area of PAH in the sample, $b$ is the intercept of the regression line, $a$ is the slope of the regression line, and $R$ is the recovery of PAH.

**Determination of PAHs in Meat Samples by HPLC.** Meat samples, including stewed chicken breast, stewed chicken wing, stewed chicken liver, stewed chicken heart, grilled chicken, grilled duck, stewed pork, stewed pork stomach, smoked Frankfurt sausage, and smoked pork, were extracted for PAHs by using Soxhlet extraction and purification with a Sep-Pak Florisil cartridge. The PAHs present in duck samples were separated, identified, and quantified by HPLC with the conditions described earlier. The following settings (excitation wavelength/emission wavelength) were used for fluorescence detection: $\lambda_1$ = 270 nm/340 nm (naphthalene, acenaphthene, fluorene); $\lambda_2$ = 254 nm/375 nm (phenanthrene); $\lambda_3$ = 260 nm/420 nm (anthracene, fluoranthene); $\lambda_4$ = 254 nm/390 nm (pyrene, benzo[*a*]anthracene, chrysene); $\lambda_5$ = 260 nm/420 nm (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene); $\lambda_6$ = 293 nm/498 nm (indeno[1,2,3-*c*,*d*]pyrene). Duplicate analyses were conducted and mean values determined.

## RESULTS AND DISCUSSION

**Comparison of Isocratic and Gradient Solvent Systems.** An isocratic binary solvent system of acetonitrile−water (70:30, v:v) with a flow rate of 2.0 mL/min and detection at 254 nm and a polymeric $C_{18}$ column were used to separate 16 PAHs as reported by Lawrence and Weber (1984b). However, the separation time was too long (60 min) and the capacity factor ($k'$) was between 0.45 and 35.64 (Table 1). In addition, some peaks (acenaphthene and fluorene) overlapped. It has been reported that $k'$ value should be controlled between 2 and 10 to achieve an ideal separation (Dolan, 1990). The author also reported that in real practice the $k'$ value can be between 1 and 20. Thus, by increasing the solvent strength of mobile phase it was possible to reduce the retention time and the $k'$ value. Nevertheless, some peaks were eluted too fast and overlapped. To remedy this problem, it was necessary to develop a gradient solvent system for the simultaneous separation of the 16 PAHs. The first system consisted of acetoni-

**Table 1. Capacity Factor ($k'$) of 16 Priority PAHs Using Isocratic and Gradient Solvent Systems with UV Detection at 254 nm**

| compound | capacity factor ($k'$) isocratic system[a] | gradient system A[b] | gradient system B[c] |
|---|---|---|---|
| naphthalene | 0.45 | 4.86 | 1.51 |
| acenaphthylene | 0.70 | 5.64 | 1.98 |
| acenaphthene | 1.17 | 6.63 | 2.72 |
| fluorene | 1.17 | 6.97 | 2.97 |
| phenanthene | 1.56 | 7.71 | 3.61 |
| anthracene | 2.08 | 8.50 | 4.35 |
| fluoranthene | 2.64 | 9.24 | 5.04 |
| pyrene | 3.34 | 9.82 | 5.63 |
| benzo[*a*]anthracene | 6.17 | 11.93 | 7.77 |
| chrysene | 7.27 | 12.48 | 8.35 |
| benzo[*a*]fluoranthene | 11.80 | 14.03 | 10.00 |
| benzo[*k*]fluoranthene | 15.32 | 14.88 | 10.95 |
| benzo[*a*]pyrene | 18.27 | 15.52 | 11.59 |
| dibenz[*a,h*]anthracene | 28.36 | 16.60 | 12.73 |
| benzo[*g,h,i*]perylene | 29.32 | 17.00 | 13.17 |
| indeno[1,2,3-*c,d*]pyrene | 35.64 | 17.57 | 13.77 |

[a] A mobile phase of acetonitrile−water (70:30, v:v) was used. [b] A mobile phase of acetonitrile−water (40:60, v:v) was maintained for 2 min, then linearly programmed to 100% acetonitrile over a 25 min period, and maintained for 13 min. [c] A mobile phase of acetonitrile−water (55:45, v:v) was maintained for 2 min, then linearly programmed to 100% acetonitrile over a 23 min period, and maintained for 15 min.

trile−water (40:60, v:v), which was maintained for 2 min in the beginning, then linearly programmed to 100% acetonitrile within 25 min, and maintained for 13 min with a flow rate of 1.25 mL/min and detection at 254 nm. The separation time for 16 PAHs was reduced to 30 min and the $k'$ value was between 4.86 (naphthalene) and 17.57 (indeno[1,2,3-*c,d*]pyrene) (Figure 1 and Table 1). This result implied that the solvent strength was not high enough to achieve an ideal $k'$ value. The first system was thus changed to acetonitrile−water (55:45, v:v), which was maintained for 2 min in the beginning, then linearly programmed to 100% acetonitrile within 23 min, and maintained for 15 min with a flow rate of 1.25 mL/min and detection at 254 nm. The $k'$ value could be reduced to between 1.51 (naphthalene) and 13.77 (indeno[1,2,3-*c,d*]pyrene) (Figure 1 and Table 1).

**Selection of an Appropriate Detection Wavelength.** The most frequently used methods for the detection of PAHs are UV and fluorescence (Wise et al., 1993). Due to the ring differences among PAHs, the selection of an appropriate wavelength for the simultaneous detection of 16 PAHs has been difficult. Table 2 shows the maximum absorption wavelengths of 16 PAHs using UV and fluorescence detection. Seven UV detection wavelengths, 230, 245, 254, 270, 295, 335, and 360 nm, were compared with respect to the peak area responses of the 16 PAHs. With wavelengths at 230, 245, and 254 nm it was found that all 16 PAHs could be simultaneously detected. Of these three wavelengths, 254 nm was adopted as the reference detection wavelength mainly because some impurities in foods such as aliphatic hydrocarbons can also be absorbed at 230 and 245 nm (Lawrence and Weber, 1984b; Gomma et al., 1993; Thompson et al., 1993). The only drawback for using 254 nm is that it resulted in low sensitivity for fluorene, fluoranthene, dibenz[*a,h*]anthracene, and benzo[*g,h,i*]perylene. To remedy this problem, it was necessary to investigate the possiblity of simultaneous detection of the 16 PAHs with maximum sensitivity by programmable fluorescence. The maximum excitation and emission wavelengths for the 16 PAHs are shown

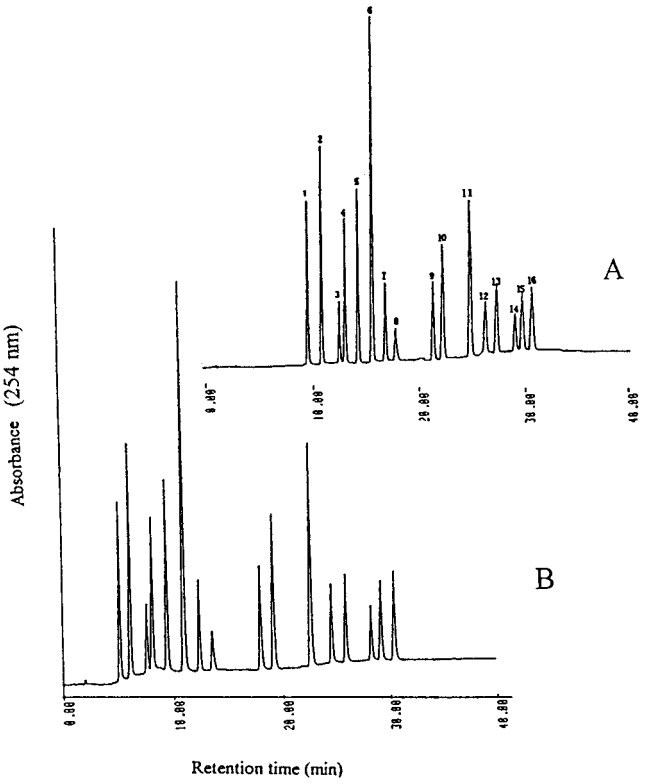


**Figure 1.** HPLC chromatograms of 16 priority PAHs employing two gradient systems with UV detection at 254 nm. Gradient A used an initial elution of acetonitrile/water (40:60, v:v) maintained for 2 min and was then linearly programmed to 100% acetonitrile over a 25 min period with a flow rate of 1.25 mL/min. Gradient B used an initial elution of acetonitrile−water (55:45, v:v) maintained for 2 min and was then linearly programmed to 100% acetonitrile over a 23 min period with a flow rate of 1.25 mL/min. See Table 2 for peak identification.

**Table 2. Maximum Absorbance Wavelengths of 16 Priority PAHs Using UV and Fluorescence Detection**

| PAH | peak no. | UV[a] (nm) | fluorescence[b] (nm) Ex | Em |
|---|---|---|---|---|
| naphthalene | 1 | 215 | 276 | 336 |
| acenaphthylene | 2 | 225 | *c* | *c* |
| acenaphthene | 3 | 223 | 292 | 340 |
| fluorene | 4 | 202 | 286 | 459 |
| phenanthrene | 5 | 246 | 250 | 366 |
| anthracene | 6 | 246 | 251 | 401 |
| fluoranthene | 7 | 206 | 261 | 315 |
| pyrene | 8 | 234 | 335 | 397 |
| benzo[*a*]anthracene | 9 | 279 | 308 | 410 |
| chrysene | 10 | 261 | 267 | 384 |
| benzo[*b*]fluoranthene | 11 | 252 | 298 | 440 |
| benzo[*k*]fluoranthene | 12 | 240 | 308 | 411 |
| benzo[*a*]pyrene | 13 | 259 | 384 | 406 |
| dibenz[*a,h*]anthracene | 14 | 290 | 296 | 396 |
| benzo[*g,h,i*]perylene | 15 | 198 | 383 | 408 |
| indeno[1,2,3-*c,d*]pyrene | 16 | 244 | 293 | 498 |

[a] UV values were obtained by using a Shimadzu SPD-M6A photodiode array UV−vis detector. [b] Excitation and emission values were obtained by using a Hitachi F-3000 fluorescence spectrophotometer. [c] Acenaphthylene has too low of a fluorescence quantum yield to be detected.

in Table 2, with the exception that no fluorescence response was observed for acenaphthylene. By comparing the various detection wavelengths at different settings (excitation wavelength/emission wavelength), it was found that 8 PAHs could be detected at 240 nm/370 nm, 5 PAHs at 270 nm/340 nm, 11 PAHs at 260 nm/420 nm, 12 PAHs at 254 nm/375 nm or 254 nm/390

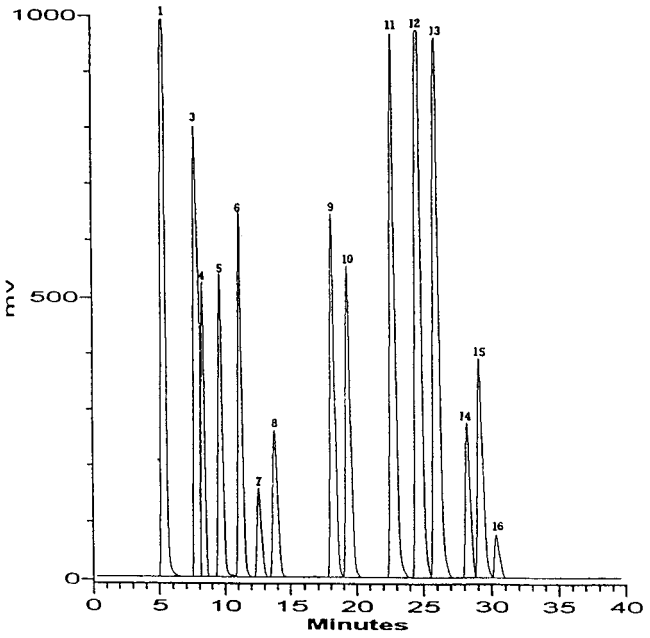


**Figure 2.** HPLC chromatograms of 15 priority PAHs employing programmed fluorescence detection. For fluorescence the following settings were used (excitation wavelength/emmission wavelength): $\lambda_1 = 270$ nm/340 nm (peaks 1−4); $\lambda_2 = 254$ nm/375 nm (peak 5); $\lambda_3 = 260$ nm/420 nm (peaks 6−7); $\lambda_4 = 254$ nm/390 nm (peaks 8−10); $\lambda_5 = 260$ nm/420 nm (peaks 11−15); $\lambda_6 = 293$ nm/498 nm (peak 16). Chromatographic conditions described in the text. See Table 2 for peak identification.

nm, and 7 PAHs at 293 nm/498 nm. Tomkins et al. (1985) determined benzo[*a*]pyrene in cigarettes with detection at 365 nm/425 nm. Takatsuki et al. (1985) quantified 10 PAHs in fish products with detection at 370 nm/410 nm. In another study, Simko and Bruncko-oya (1993) determined six PAHs in liquid flavor smoke with detection at 310 nm/410 nm. From the preceding discussion, it was found that the selection of a single fluorescence wavelength failed to detect 16 PAHs simultaneously. Thus, it is necessary to employ programmable fluorescence instead. The following settings were used: $\lambda_1 = 270$ nm/340 nm (naphthalene, acenaphthene, fluorene); $\lambda_2 = 254$ nm/375 nm (phenanthrene); $\lambda_3 = 260$ nm/420 nm (anthracene, fluoranthene); $\lambda_4 = 254$ nm/390 nm (pyrene, benzo[*a*]anthracene, chrysene); $\lambda_5 = 260$ nm/420 nm (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene); $\lambda_6 = 293$ nm/498 nm (indeno[1,2,3-*c*,*d*]pyrene). Figure 2 shows the HPLC chromatograms of 15 PAHs using programmable fluorescence. Table 3 shows the detection limits of 16 PAHs using UV and fluorescence detection. The detection limits for the former and the latter were 0.03−1.54 ng and 0.5−6.0 pg, respectively. This result indicated that fluorescence detection had about 20−320 times higher sensitivity than UV detection. Similar results were observed in some other reports (Dong and Greenberg, 1988; Dong et al., 1993; Wise et al., 1993).

**Comparison of Extraction Methods.** Table 4 shows the effect of various elution solvents on the recoveries of PAHs of grilled duck *n*-hexane extract using the Sep-Pak Florisil cartridge. The recoveries of most PAHs eluted with 10 mL of hexane and 8 mL of hexane−methylene chloride (3:1, v:v) (solvent system A) were significantly higher ($p < 0.05$) than those eluted with 18 mL of hexane−methylene chloride (3:1, v:v)-(solvent system B). The latter also failed to elute PAHs containing five or six rings. This can be attributed to the fact that the solvent strength of hexane−methylene chloride (3:1, v:v) was too low to elute all PAHs from the Sep-Pak Florisil cartridge. In a similar study, Karlesky et al. (1986) found that PAHs could not be eluted by 100% methylene chloride; instead, most PAHs could be eluted by cyclohexane−methylene chloride (97:3, v:v).

Both HPLC chromatograms of PAHs in grilled duck extracted by the Soxhlet method with and without partition procedures showed almost the same number of peaks, indicating that partition did not remove impurities significantly. Nevertheless, fluorescence detection was found to have fewer peaks than UV detection, implying that some impurities detected by UV did not produce fluorescence. It has been reported that the partition procedure can effectively remove some disturbed substances, such as aliphatic hydrocarbons, fatty acids, phenol, polycyclic organic substances (Wise et al., 1977; Natusch and Tomkins, 1978). During partition aliphatic hydrocarbon can be partitioned into the cyclohexane layer, so that PAHs then form complexes with DMSO through linkage of the electron-rich portion of the $\pi$ ring and the lone-pair electrons of the sulfur atom. After the addition of cyclohexane and water, DMSO and water were partitioned into the lower layer while PAHs partitioned into the cyclohexane layer. Some polar compounds such as phenol and free fatty acid can be hydrogen-bonded with DMSO and, thus, can

**Table 3. Detection Limits of 16 Priority PAHs Using UV and Fluorescence Detection[a]**

| | | UV | | programmed fluorescence | | |
|---|---|---|---|---|---|---|
| PAH | peak no. | detection limit (ng) | wavelength (nm) | detection limit (pg) | Ex/Em (nm) | UV/FL ratio |
| naphthalene | 1 | 0.6 | 254 | 5 | 270/340 | 120 |
| acenaphthylene | 2 | 0.93 | 254 | ND | 270/340 | |
| acenaphthene | 3 | 1.54 | 254 | 6 | 270/340 | 257 |
| fluorene | 4 | 0.16 | 254 | 2 | 270/340 | 80 |
| phenanthrene | 5 | 0.06 | 254 | 1 | 254/370 | 60 |
| anthracene | 6 | 0.03 | 254 | 0.8 | 260/420 | 37.5 |
| fluoranthene | 7 | 0.36 | 254 | 6 | 260/420 | 60 |
| pyrene | 8 | 0.4 | 254 | 2 | 254/390 | 200 |
| benzo[*a*]anthracene | 9 | 0.8 | 254 | 0.8 | 254/390 | 125 |
| chrysene | 10 | 0.08 | 254 | 1 | 254/390 | 80 |
| benzo[*b*]fluoranthene | 11 | 0.1 | 254 | 1 | 260/420 | 100 |
| benzo[*k*]fluoranthene | 12 | 0.16 | 254 | 0.5 | 260/420 | 320 |
| benzo[*a*]pyrene | 13 | 0.16 | 254 | 0.5 | 260/420 | 320 |
| dibenz[*a*,*h*]anthracene | 14 | 0.4 | 254 | 3.4 | 260/420 | 118 |
| benzo[*g*,*h*,*i*]perylene | 15 | 0.3 | 254 | 2.4 | 260/420 | 125 |
| indeno[1,2,3-*c*,*d*]pyrene | 16 | 0.12 | 254 | 6 | 293/498 | 20 |

[a] Detection limit determined at S/N = 3. ND, not detected.

**Table 4. Influence of Various Elution Solvents on Recoveries of PAHs of Grilled Duck Meat *n*-Hexane Extract Using Sep-Pak Florisil Cartridge**

| compound | recovery (%)[c] elution solvent A[d] | | elution solvent B[d] | |
|---|---|---|---|---|
| naphthalene | 63.0 [a] | (2.24)[b] | 92.5[1] | (0.76) |
| acenaphthylene | 73.0 | (3.87) | 92.0[1] | (1.54) |
| acenaphthene | 75.5 | (2.81) | 83.0[1] | (1.70) |
| fluorene | 80.5[1] | (4.39) | 83.5[1] | (2.54) |
| phenanthrene | −[2 e] | − | 77.5 | (2.74) |
| anthracene | 9.0[2] | (15.71) | 87.0[1] | (1.63) |
| fluoranthene | 44.0[2] | (9.64) | 77.0[1] | (1.84) |
| pyrene | − | − | 73.5[1] | (2.89) |
| benzo[*a*]anthracene | − | − | 82.5[1] | (0.86) |
| chrysene | − | − | 90.0[1] | (1.57) |
| benzo[*b*]fluoranthene | − | − | 81.5[1] | (2.60) |
| benzo[*k*]fluoranthene | − | − | 84.0[1] | (5.05) |
| benzo[*a*]pyrene | − | − | 95.0[1] | (4.47) |
| dibenz[*a*,*h*,*i*]perylene | − | − | 103.5[1] | (6.15) |
| benzo[*g*,*h*,*i*]perylene | − | − | 110.5[1] | (9.60) |
| indeno[1,2,3-*c*,*d*]pyrene | − | − | 94.5[1] | (2.24) |

[a] Different superscript numbers in the same row are significantly different ($p < 0.05$). [b] Values in parentheses represent coefficient of variation (%). [c] Mean of duplicate determinations. [d] Elution solvent A consists of 10 mL of hexane followed by 8 mL of hexane−methylene chloride (3:1, v:v), while elution solvent B consists of 18 mL of hexane−methylene chloride (3:1, v:v). [e] Not recovered by elution solvent A.

be removed from the cyclohexane layer. From the preceding discussion, it can be concluded that partition is not necessary during the extraction of PAHs from meat products. A similar result was observed by Karlesky et al. (1986), who purified PAHs from air particulate samples by DMSO−cyclohexane partition and solid phase extraction and found that the latter resulted in higher recovery than the former.

Table 5 shows the recoveries of PAHs spiked to grilled duck by Soxhlet and sonication extraction. With Soxhlet extraction, grilled duck was found to contain naphthalene, phenanthrene, benzo[*a*]anthracene, and chrysene. On the contrary, with sonication grilled duck was found to contain naphthalene and phenanthrene. This is probably because some PAHs in duck meat can form complexes with lipid or protein, and solvents such as hexane or methylene chloride may fail to extract PAHs from the matrix by sonication. Grimmer and Bohnke (1975) pointed out that saponification is necessary for the extraction of PAHs from meat or fish products. The authors also found that only 30% of benzo[*a*]pyrene and other PAHs were extracted from fish by methanol. However, with saponification about 90% PAHs can be extracted. Thompson et al. (1993) reported that saponification with methanolic potassium hydroxide for 2 h could extract a large amount of PAHs from oysters. In our study, the recoveries of PAHs were in the range of 72.0−101.5% for Soxhlet extraction. With sonication and hexane or methylene chloride as the extracting solvent, the recoveries of PAHs were 73.5−110.5% for the former and 54−95.5% for the latter. It was also found that the former method resulted in lower recovery of naphthalene, acenaphthylene, dibenz[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perylene. This is probably because naphthalene and acenaphthylene are semivolatile and were evaporated during concentration under vacuum (Karlesky et al., 1986; Dong et al., 1993). In addition, both dibenz[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]perylene may undergo partial loss during the purification of PAHs by the Sep-Pak Florisil cartridge. Lawrence and Weber (1984b) reported that the low recovery of PAHs correlated well to the purification step by a Florisil column. It is also possible that light can degrade PAHs during extraction (Gomma et al., 1993). From the preceding discussion, it can be concluded that sonication is not applicable for the extraction of meat products because no saponification was employed. Nevertheless, extraction time and solvent consumption can be greatly reduced by the sonication method. This method can also be applied to other types of samples such as plant tissue, soil, and water (Dong et al., 1993; Chuang et al., 1995).

**Determination of PAHs in Commercial Meat Products.** Table 6 shows the amounts of various PAHs in some commercial meat products, including stewed pork, stewed pork stomach, stewed Frankfurt sausage, smoked pork, stewed chicken breast, stewed chicken wing, stewed chicken liver, grilled chicken, and grilled duck. The major PAHs present in meat products included naphthalene, phenanthrene, benzo[*a*]anthracene, and chrysene. The lowest PAH concentrations were found in stewed chicken breast, with 1.5 ppb naphthalene and 0.1 ppb phenanthrene, stewed pork, with 0.9 ppb naphthalene, 1.3 ppb phenanthrene, 0.4 ppb pyrene, and 0.5 ppb chrysene. The highest PAH concentrations were found in grilled duck, with 55.2 ppb naphthalene, 3.9 ppb phenanthrene, 10.2 ppb benzo[*a*]-anthracene, and 2.7 ppb chrysene, and stewed chicken liver, with 77.6 ppb naphthalene, 0.5 ppb anthracene, 25.3 ppb fluoranthene, 26.6 ppb benzo[*a*]anthracene 8.5

**Table 5. Recoveries (%)[c] of PAHs Spiked to Grilled Duck Meat by Various Extraction Methods**

| compound | Soxhlet extraction | | sonication extraction *n*-hexane | | sonication extraction dichloromethane | |
|---|---|---|---|---|---|---|
| naphthalene | 78.5[2 a] | (4.50)[b] | 92.5[1] | (0.76) | 95.5[1] | (0.74) |
| acenaphthylene | 82.5[2] | (0.86) | 92.0[1] | (1.54) | 91.5[1] | (0.77) |
| acenaphthene | 80.5[1] | (4.39) | 83.0[1] | (1.70) | 89.5[1] | (0.79) |
| fluorene | 80.5[1] | (2.64) | 83.5[1] | (2.54) | 89.5[1] | (2.37) |
| phenanthrene | 90.0[1] | (1.57) | 77.5[2] | (2.74) | 74.5[1] | (2.85) |
| anthracene | 99.0[1] | (2.86) | 87.0[1] | (1.63) | 74.0[2] | (3.82) |
| fluoranthene | 85.5[1] | (0.83) | 77.0[1,2] | (1.84) | 68.0[2] | (4.16) |
| pyrene | 91.0[1] | (3.11) | 73.5[2] | (2.89) | 69.0[2] | (2.05) |
| benzo[*a*]anthracene | 80.5[1] | (4.39) | 82.5[1] | (0.86) | 75.5[1] | (4.68) |
| chrysene | 101.5[1] | (0.70) | 90.0[1] | (1.57) | 75.0[2] | (7.54) |
| benzo[*b*]fluoranthene | 96.5[1] | (2.20) | 81.5[2] | (2.60) | 72.0[3] | (1.96) |
| benzo[*k*]fluoranthene | 92.0[1] | (1.54) | 84.0[1] | (5.05) | 68.5[2] | (1.03) |
| benzo[*a*]pyrene | 86.5[1] | (2.45) | 95.0[1] | (4.47) | 62.0[2] | (2.28) |
| dibenz[*a*,*h*]perylene | 72.0[2] | (3.93) | 103.5[1] | (6.15) | 54.0[2] | (2.62) |
| benzo[*g*,*h*,*i*]perylene | 74.5[2] | (4.75) | 110.5[1] | (9.60) | 58.0[2] | (2.44) |
| indeno[1,2,3-*c*,*d*]pyrene | 76.0[1,2] | (5.58) | 94.5[1] | (2.24) | 66.0[2] | (6.43) |

[a] Different superscript numbers in the same row are significantly different ($p < 0.05$). [b] Values in parentheses represent coefficient of variation (%). [c] Mean of duplicate determinations.

**Table 6. PAH Concentrations (ppb) in Selected Commercial Meat Products[a]**

| PAH | stewed pork | stewed pork stomach | smoked Frankfurt sausage | smoked pork | stewed chicken breast | stewed chicken wing | stewed chicken liver | stewed chicken heart | grilled chicken | grilled duck |
|---|---|---|---|---|---|---|---|---|---|---|
| naphthalene | 0.9 | 19.1 | 12.0 | 0.8 | 1.5 | 36.6 | 77.6 | 2.9 | 19.9 | 55.2 |
| acenaphthylene | ND[c] | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| acenaphthene | ND | ND | ND | ND | ND | 0.6 | ND | ND | ND | ND |
| fluorene | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| phenanthrene | 1.3 | 1.1 | 0.7 | 1.8 | 0.1 | 3.7 | ND | 1.1 | 2.1 | 3.9 |
| anthracene | ND | ND | ND | 0.2 | ND | 0.3 | 0.5 | ND | ND | ND |
| fluoranthene | ND | ND | ND | 0.2 | ND | ND | 25.3 | ND | ND | ND |
| pyrene | 0.4 | ND | 0.1 | ND | ND | ND | ND | ND | ND | ND |
| benzo[*a*]anthracene* [b] | ND | 2.2 | 1.6 | 2.8 | ND | ND | 26.6 | 10.2 | 31.8 | 10.2 |
| chrysene | 0.5 | 1.0 | 0.5 | 1.4 | ND | ND | 8.5 | 3.4 | ND | 2.7 |
| benzo[*b*]fluoranthene* | ND | ND | ND | 0.1 | ND | ND | 1.4 | ND | 1.8 | ND |
| benzo[*k*]fluoranthene | ND | ND | ND | 0.2 | ND | ND | 1.6 | 1.4 | ND | ND |
| benzo[*a*]pyrene* | ND | ND | ND | 0.1 | ND | ND | ND | ND | 4.6 | ND |
| dibenz[*a*,*h*]anthracene* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| benzo[*g*,*h*,*i*]perylene | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| indeno[1,2,3-*c*,*d*]pyrene* | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| total of PAHs | 3.1 | 23.4 | 14.9 | 7.5 | 1.6 | 41.2 | 141.5 | 19.0 | 60.2 | 72.0 |
| total of carcinogenic PAHs | ND | 2.2 | 1.6 | 3.0 | ND | ND | 28.0 | 10.2 | 38.2 | 10.2 |

[a] Mean of duplicate analyses. [b] * indicates carcinogenic PAH. [c] ND, not detectable.

ppb chrysene, 1.4 ppb benzo[*b*]fluoranthene, and 1.6 ppb benzo[*k*]fluoranthene. The total concentrations of carcinogenic PAHs, including benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and indeno[1,2,3-*c*,*d*]pyrene, were found in stewed chicken liver at 28.0 ppb and in grilled chicken at 38.2 ppb. No carcinogenic PAHs were observed for stewed pork, stewed chicken breast, and stewed chicken wing. From the preceding results, it may be postulated that the processing of meat by smoking and grilling resulted in higher concentrations of carcinogenic PAHs. Lawrence and Weber (1984a) determined PAHs in smoked fish and found that they were present at a higher concentration in smoked fish than in nonsmoked fish. Tuominen et al. (1988) found that smoked cereal contained a 100 times higher concentration of benzo[*a*]pyrene than nonsmoked cereal. Apparently the difference in the formation of a specific PAH during smoking can be attributed to heating time, temperature, variety of smoked wood, and fat content of product (Vaessen et al., 1988). In addition to smoked products, grilled chicken and duck also contained high concentrations of PAHs. This is probably because during grilling fat could be dropped onto charcoal, and some PAHs formed through heating might be evaporated along with the fat and adhered to the meat surface. It has been reported that the amount of PAHs formed during charcoal-broiling of meat can be attributed to the amount of fat originally present in the meat, the closeness of meat to the heat source, and the exposure time of the meat to heat (Lijinsky and Ross, 1967; Doremire et al., 1979). Gomma et al. (1993) further reported that grilled pork chop contained higher amounts of PAHs than smoked ham and sausage. Of the various meat samples investigated in this study, stewed chicken liver and stewed chicken heart were found to contain high amounts of carcinogenic PAHs. These data indicated that PAHs might be deposited in the guts of chicken. Similar results were reported by Dunn and Fee (1979) and Lawrence and Weber (1984b), who found that the digestive organs of poultry could deposit high amounts of PAHs. In contrast, in another study Lawrence and Weber (1984a) found that PAHs could not be deposited in the digestive organs of fish. Since these results are contradictory, the deposition of PAHs in animal body needs to be investigated further.

In conclusion, a gradient solvent system was developed to separate 16 PAHs with UV and programmable fluorescence detection. Saponification and partition are necessary for the extraction of PAHs from meat products by UV detection. With fluorescence detection partition can be discarded. Both grilled and smoked meats were found to contain high amounts of PAHs. The method developed in this study can be applied to the determination of PAHs in processed meat products.

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