SFE-plus-C_{18} Lipid Cleanup and Selective Extraction Method for GC/MS Quantitation of Polycyclic Aromatic Hydrocarbons in Smoked Meat

Md. Yeakub Ali and Richard B. Cole*

Department of Chemistry, University of New Orleans, 2000 Lakeshore Drive, New Orleans, Louisiana 70148

In biological matrixes lipid material often poses an interference problem for determinations of nonpolar compounds, e.g., polycyclic aromatic hydrocarbons (PAHs). A newly developed supercritical fluid extraction plus adsorbent method, "SFE-plus-C_{18}", offers selective extraction of PAHs in lipid-rich biological matrixes without the need for supplementary cleanup. This method eliminates the use of large volumes of toxic solvent and lengthy lipid removal procedures. This study reports the first application of the SFE-plus-C_{18} method to the analysis of a genuine food product, i.e., smoked meat (beef). The procedure employs the addition of C_{18} adsorbent beads to the initial sample slurry of pureed smoked meat prior to supercritical CO_2 extraction and GC/MS quantitation. During SF extraction, indigenous lipids are preferentially retained on the beads, and PAHs are selectively extracted with supercritical CO_2. In a comparison of determinations of PAHs by SFE-plus-C_{18} vs the conventional SFE method, only 11−17% of the indigenous lipids observed by the conventional SFE method were co-extracted using the SFE-plus-C_{18} method. The PAHs in smoked meat could thus be determined efficiently in the presence of a reduced background of co-extracted lipids. Out of 10 targeted PAHs, seven were detected with a range of 10.0−26.0 ng/g in the smoked meat sample. The other three PAHs were not present above the detection limit of the instrument (2.5−4.1 pg). The recoveries of PAHs obtained using the conventional SFE method were 63−94% lower than those achieved by SFE-plus-C_{18}.

Keywords: *Supercritical fluid extraction; lipid cleanup; polycyclic aromatic hydrocarbons; smoked meat*

INTRODUCTION

Polycylic aromatic hydrocarbons (PAHs) are environmentally hazardous organic compounds because of their known or suspected carcinogenicity (*1*−*3*). Hence, the determination of PAHs in foods such as meat and fish is important for human safety. Furthermore, these environmentally persistent nonpolar pollutants have a strong tendency to accumulate in the lipid material of ingesting organisms, including humans, via PAH-contaminated foodstuff. This has prompted the continued refinement of methodologies to accurately measure the level of PAHs in the environment, especially in water and in foods.

The analysis of PAHs in fish (*4*), seafood (*5*, *6*), meat (*7*−*9*), and chicken eggs (*10*) usually involves a liquid solvent extraction step (e.g., Soxhlet extraction) followed by a multistep cleanup and separation procedure. Such techniques are labor intensive and consume large volumes of costly and toxic solvents (e.g., dichloromethane, benzene). In the past decade, supercritical fluid extraction (SFE) has gained in popularity as a suitable technique for environmental sample analyses (*11*−*21*). SFE employing supercritical CO_2 has several distinct advantages over traditional solvent extraction techniques including faster speed, adjustable solvent power, extremely low toxicity, and smaller sample volumes (*22*−*24*).

There have been several reports in recent years wherein SFE was used for investigations of low-polarity constituents such as polychlorinated biphenyls or pesticides present in lipid-containing matrixes (*25*−*38*). In those studies, lipids were always co-extracted with the low-polarity analytes of interest. The extracts were then subjected to a cleanup step to remove lipids prior to analyses. In these cases, solid-phase adsorbent traps (*39*, *40*) or columns packed with alumina, silica, florisil, or C_{18} (octadecyl siloxane) beads were used to remove coextracted lipids from the extract using either on-line or off-line means after SFE was performed. Alternative sorbents, such as basic alumina (*26*, *33*, *34*) or activated carbon (*35*), have also been used in the SFE extraction chamber to remove lipids from the extracts of spiked samples. Barker and co-workers (*41*−*44*) have used C_{18} as a nonpolar adsorbent in a variety of biological matrixes employing the technique of matrix solid-phase dispersion (MSPD). In these studies, C_{18} beads were used to adsorb nonpolar to slightly polar analytes from matrixes such as milk (*42*, *43*), animal fat (*44*), and animal tissues (*41*). Recently, Amigo et al. (*45*) reported extraction of PAHs from bird (*Tyto alba*) liver. After SFE, cleanup by a reversed-phase C_{18} precolumn was performed prior to chromatographic determination in this latter study.

We have reported a first example of a novel sample preparation method called SFE-plus-C_{18} (*46*, *47*). This

* To whom correspondence should be addressed. Telephone: (504)280-6311. Fax: (504)280-6860. E-mail: rcole@uno.edu.

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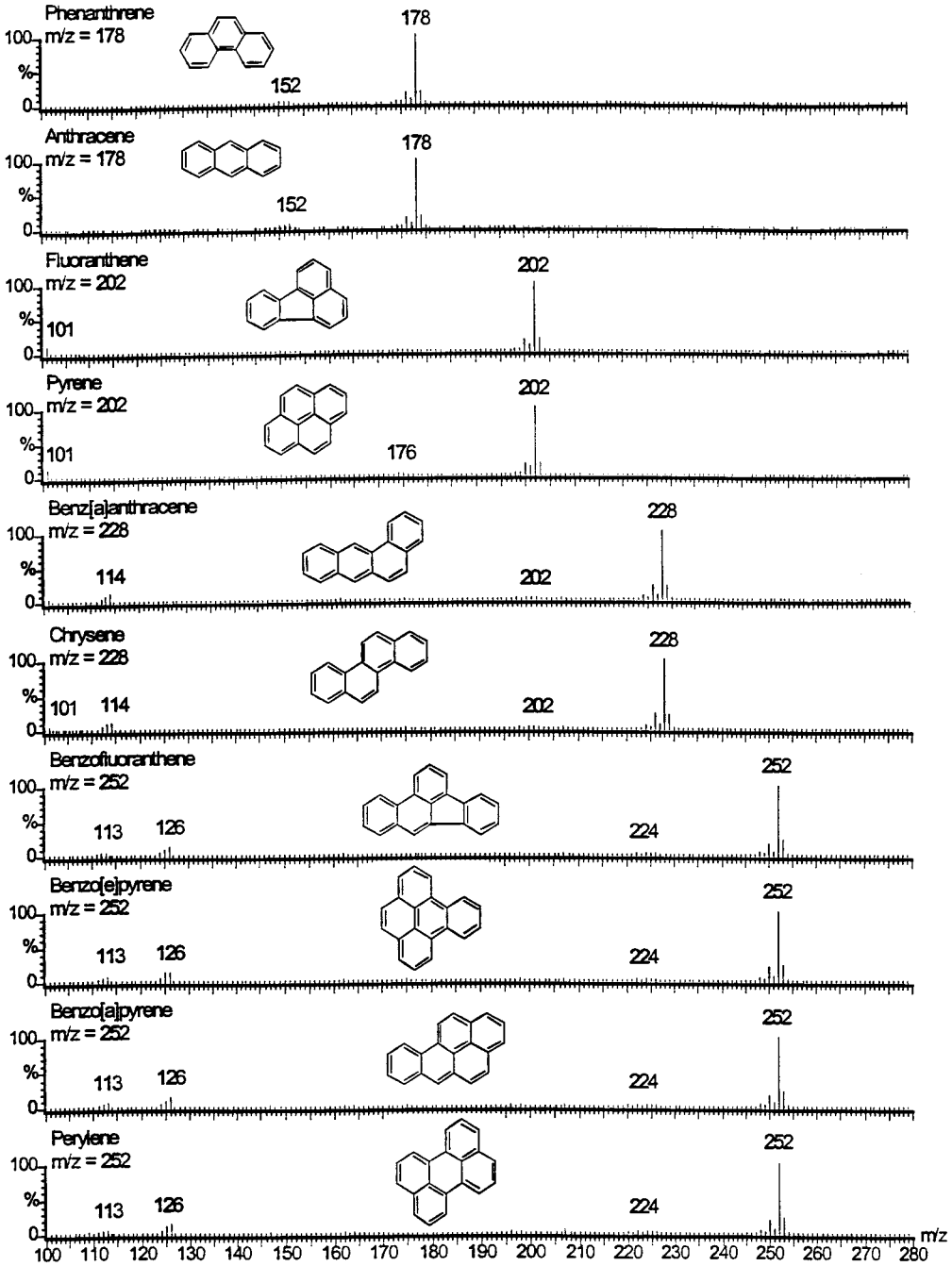

Figure 1. Mass spectra and molecular structures of ten investigated PAH standards.

method uses C_{18} as a nonpolar (silaceous) adsorbent bead to trap lipid materials inside the SFE chamber during extraction with supercritical CO_2. The method was shown to allow selective extraction of PAHs in lipid-containing matrixes, i.e., spiked crab tissue. Whereas the previous study (*46*, *47*) introduced the SFE-plus-C_{18} method and established its potential using spiked contaminants, the current study is the first report on applying the method to the analyses of actual foods in their consumer product form. The current report expands the application of the method to smoked meat, a high-lipid-content foodstuff, for the purpose of direct quantification of PAHs by GC/MS. Longer exposure times of meat to smoke during preparation has been shown previously to correlate with higher PAH content (*9*). Ten carcinogenic or suspect carcinogenic PAHs were selected as target analytes of interest.

MATERIALS AND METHODS

Preparation of PAH Standards. High purity (>99%) solid PAHs (Figure 1) were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of the individual PAHs were prepared, and subsequent serial dilutions in analytical grade methylene chloride (EM Science, Gibbstown, NJ) were performed to prepare analytical standards, including mixed standards that were all stored before use at −5 °C.

Pretreatment of C_{18} Beads. Analytical grade C_{18} nonpolar adsorbent beads (35−75 μm size, 60 Å porosity), purchased from Alltech Associates, Inc. (Deerfield, IL), were washed sequentially with at least two bed volumes of hexane, methanol, and methylene chloride and were then dried and also stored at room temperature. A total of 100 g of beads was prepared, and 2 g was used for each extraction.

Smoked Meat. A name-brand smoked meat sample (beef) was purchased from a local New Orleans market. About 100 g of meat from several packets was removed and then pureed

Table 1. Instrument Detection Limits for Ten Target PAHs and Correlation Coefficients of Calibration Curves for the Same Ten PAH Standards

target PAH	detection limit in pg	correlation coefficient (r)
phenanthrene	2.9 ± 0.6	0.990
anthracene	2.9 ± 0.5	0.990
fluoranthene	2.8 ± 0.5	0.986
pyrene	2.5 ± 0.4	0.999
benzo[*a*]anthracene	3.9 ± 0.8	0.999
chrysene	3.8 ± 0.4	0.999
benzofluoranthene	3.9 ± 0.6	0.975
benzo[*e*]pyrene	4.1 ± 0.7	0.993
benzo[*a*]pyrene	4.1 ± 0.8	0.993
perylene	4.0 ± 0.8	0.997

in a blender until smooth solid mixtures were obtained. The pureed meat was stored at −30 °C until needed.

SFE. A Hewlett-Packard model HP 7680A (Palo Alto, CA) supercritical fluid extractor was used for all SFE work. A mixture of 2.0 g of C_{18} beads or filter paper, and 0.5 g of the pureed samples, was subjected to extraction with supercritical CO_2. The SFE was performed at 100 °C and 350 bar utilizing 5 min of static equilibration followed by dynamic extraction at 1.5 mL/min for 25 min. Three methylene chloride rinses (each 1.0 mL) of the stainless steel trapping beads (held at 30 °C during extractions) allowed quantitative transfer of the extracted compounds to 1.5-mL glass receiver vials.

GC/MS. A magnetic sector GC/MS instrument (Fisons 8000 GC coupled to an Autospec tandem MS equipped with an Opus data system; Micromass, Inc. Manchester, UK) was used for all determinations in this study. A 30 m × 0.32 mm i.d. × 0.25 μm film thickness, BPX-5 5% phenyl siloxane (SGE) fused-silica capillary was used throughout as the GC column. The GC column was temperature programmed as follows: 1 min isothermal at 70 °C, then raised 8 °C/minute to 280 °C, and finally held isothermal at 280 °C for 8 min. Helium gas served as the carrier gas at a flow rate of ∼3−4 mL/min. Splitless injections were performed with the injection port heated to 280 °C. The magnetic sector mass spectrometer was operated in the electron ionization (EI) mode (electron energy 70 eV, source temperature 150 °C) scanning from m/z 50 to 450 at 1.6 s/scan. The instrument was calibrated with perfluorokerosene, with a resolution of 1000 (m/Δm) at m/z 219 on each day before acquiring data.

Standard calibration curves were generated each day that samples were run. Table 1 lists the correlation coefficients of the individual compound calibration curves, along with the detection limits for each PAH standard. All smoked meat samples were extracted in triplicate, and each extract was injected into the GC/MS instrument three times, so that a total of nine determinations was made for each sample. For 1.0-μL GC/MS injections, the instrumental limit of detection limit for the range of PAHs was 2.5−4.1 pg (i.e., 2.5−4.1 pg/μL injected). The signals corresponding to PAH molecular ions ($M^{+\bullet}$) were used for quantitation. All MS data acquired on the Opus data system were transferred to a Masslynx 3.0 (Micromass) data system. Quantitation of all data was done by Masslynx 3.0 and the results appear in Tables 2 and 3.

Signal Suppression Study. Standard solutions of *n*-tetradecanoic (myristic) acid (20, 100, 500, 2500, and 5000 ng/μL) were prepared by serial dilutions in methylene chloride along with a blank. Each of the solutions was also prepared to be 20 ng/μL in phenanthrene. A 1-μL portion of solution was allowed to evaporate in a direct-insertion sample cup. An EI mass spectrum of the dried contents of each solution was obtained using the direct introduction (solids) sample probe.

RESULTS AND DISCUSSION

The goal of this study is to expand the application of the SFE-plus-C_{18} method to a high-lipid-containing genuine food sample (i.e., a commercially available smoked meat) to determine nonpolar compounds such as PAHs without the need for sample cleanup. Nonpolar lipids are potentially deleterious in determinations of trace level PAHs because lipid materials not only mask analyte peaks, but they also degrade the GC column irreversibly. The purpose of the addition of C_{18} nonpolar adsorbent directly to the sample slurry prior to SFE is to preferentially adsorb lipid material onto the C_{18} matrix while selectively extracting PAHs using supercritical CO_2. The entire sample mixture, including C_{18} adsorbent, is placed in the extraction chamber and subjected to SFE conditions. Ten PAHs that are carcinogens or suspect carcinogens were chosen as analytes of interest in this study. Figure 1 lists the PAHs in order of their chromatographic elution on the GC column (m/z values for the intact molecular ions, $M^{+\bullet}$, are given in parentheses): phenanthrene (m/z 178), anthracene (m/z 178), fluoranthene (m/z 202), pyrene (m/z 202), benz[*a*]-anthracene (m/z 228), chrysene (m/z 228), benzofluoranthene (m/z 252), benzo[*e*]pyrene (m/z 252), benzo[*a*]-pyrene (m/z 252), and perylene (m/z 252). The molecular structures and the EI mass spectra of the selected PAH standards are also given in Figure 1.

In applying the SFE-plus-C_{18} method to the smoked meat sample to determine the level of PAHs contained in it, samples of finely ground smoked meat were directly mixed with C_{18} adsorbent beads. Three replicates of the homogenized (mixed with a mortar and pestle) mixtures were extracted by SFE under optimized conditions (100 °C, 350 bar CO_2; *47*). Extracts were then directly run by GC/MS without any additional treatment or cleanup. A similar procedure was followed in performing a conventional SFE extraction (control) except that inert filter paper replaced the C_{18} adsorbent.

Figure 2 shows a comparison of the total ion chromatograms (TIC) of the extracts of the smoked meat sample obtained from the conventional SFE method vs the SFE-plus-C_{18} method. The peaks labeled with letters correspond to the indigenous lipids; lipid "a" at m/z 228 has been assigned as *n*-tetradecanoic (myristic) acid as confirmed by matching its EI mass spectrum with that of an authentic standard. Lipids "c" (m/z 256) and "e" (m/z 284) have been assigned as *n*-hexadecanoic (palmitic) acid and *n*-octadecanoic (stearic) acid, respectively, according to very close matches with EI mass spectra found in a compiled database (John Wiley & Sons, NY). PAHs are not readily visible in the TIC because the PAHs are present at very minor levels compared to those of the lipids. From integration of the area of the peaks given in Figure 2A with the presumption of roughly equivalent responses for the various lipid components, it can be deduced that lipids "c" (*n*-hexadecanoic acid at m/z 256), "d" (m/z 264), and "e" (*n*-octadecanoic acid at m/z 284) are present at the highest concentrations. Here the lipids give very high background signals using the conventional SFE method (Figure 2A). In contrast (Figure 2B), the lipids were extracted to a much lesser extent using the SFE-plus-C_{18} method.

The signal intensities of indigenous lipids from the smoked meat sample obtained by SFE-plus-C_{18} are compared to those obtained by the conventional method in Table 2. For the extract obtained by the SFE-plus-C_{18} method, the signal of *n*-hexadecanoic acid (lipid "a") is reduced to 11.2% compared to that of the conventional SFE method, and lipid "b" was not detected. The signal of *n*-hexadecanoic acid (lipid "c"), the lipid exhibiting the

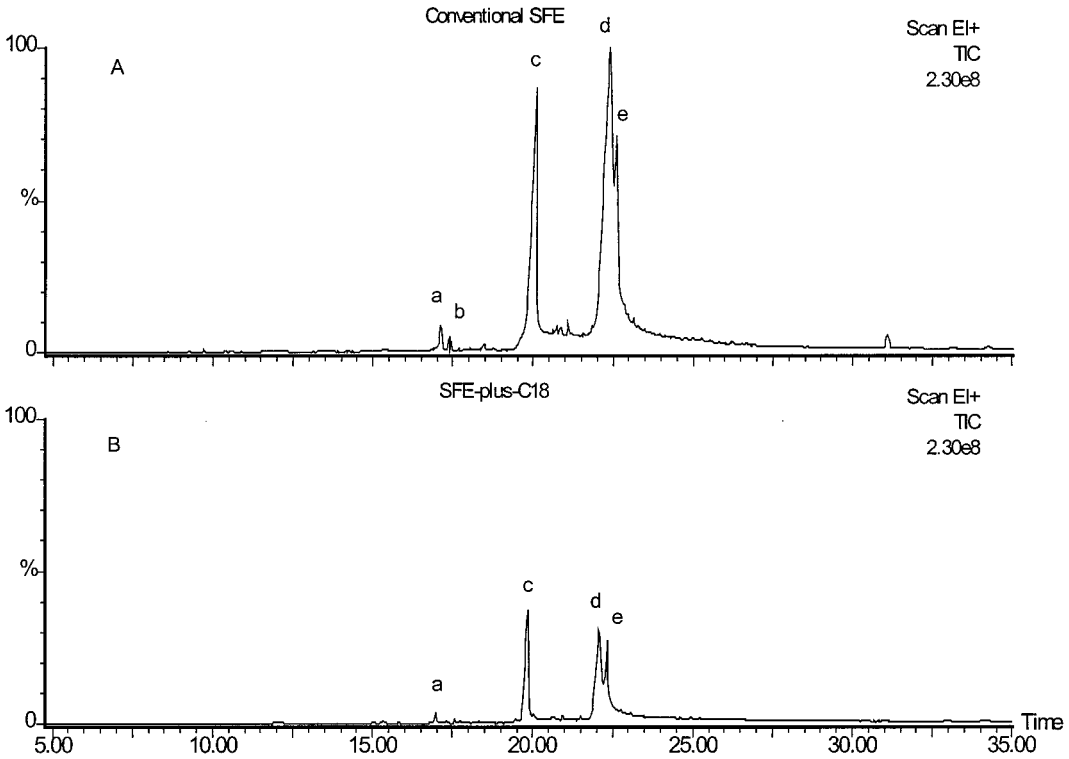

Figure 2. GC/MS total ion chromatograms of extracts from pureed smoked meat sample. A: conventional SFE (using filter paper as an inert sorbent). B: SFE-plus-C_{18}. Peaks labeled with letters correspond to various indigenous lipids: "a" (*m*/*z* 228), "b" (*m*/*z* 222), "c" (*m*/*z* 256), "d" (*m*/*z* 264), and "e" (*m*/*z* 284). Note that the full-scale intensities of Figures 2A and 2B are identically scaled to enable direct comparison of results.

Table 2. Relative Signal Intensities of Lipids in Smoked Meat Samples by SFE Methods

	signal intensity of lipids (mean ± SD)	
compound	conventional SFE[a]	SFE-plus-C_{18}[b]
lipid "a" (*m*/*z* 228)	100.0 ± 13.8	11.2 ± 3.7
lipid "b" (*m*/*z* 222)	100.0 ± 11.7	not detected
lipid "c" (*m*/*z* 256)	100.0 ± 9.1	17.4 ± 4.1
lipid "d" (*m*/*z* 264)	100.0 ± 12.3	13.5 ± 3.5
lipid "e" (*m*/*z* 284)	100.0 ± 10.3	16.0 ± 3.2

[a] Average values are assigned as 100.0. Standard deviation gives an indication of run to run variability. [b] Values are relative to assignment of 100.0 by conventional SFE method for same sample.

highest GC/MS response, was reduced to 17.4%. Table 2 therefore serves to illustrate that the lipids were coextracted to a much lesser extent during the SFE-plus-C_{18} method, only 11.2−17.4%, as compared to the conventional method. In other words, the use of the integrated adsorbent removed 82.6−88.8% of the lipids during the SFE. The above results demonstrate that SFE-plus-C_{18} is quite effective at removing lipids present in the smoked meat extract.

Figure 3 shows the summed selected ion chromatograms of *m*/*z* 178, 202, 228, and 252 which encompasses all 10 target PAH compounds, some of which are isomeric and thus appear at the same *m*/*z* values (see Figure 1). The extracts obtained by using the conventional SFE method appear in Figure 3A, while those obtained by using the SFE-plus-C_{18} method are shown in Figure 3B. Note that each of the *Y*-axes in Figure 3 is magnified by a factor of 124 relative to those in Figure 2. The peaks labeled with numbers (1−7) correspond to PAHs in the smoked meat sample. The peaks labeled with letters (i.e., "a" and "c", labeling consistent with Figure 2) correspond to indigenous lipids in the same sample. One peak represents the molecular ion of *n*-tetradecanoic acid (lipid "a") and the other corresponds to an EI fragment of *n*-hexadecanoic acid (lipid "c") containing one ^{13}C atom. Appearing at the same retention time as this latter peak is the analogous, but larger, *n*-hexadecanoic acid fragment peak containing only ^{12}C (at *m*/*z* 227, not shown) as well as the intact molecular ion of *n*-hexadecanoic acid. The molecular ion of *n*-tetradecanoic acid (lipid "a") and the above-described fragment of *n*-hexadecanoic acid (lipid "c") both appear at *m*/*z* 228 and are thus isobaric with (i.e., have the same nominal *m*/*z* value as) benz[*a*]anthracene and chrysene. In the top chromatogram (conventional SFE, Figure 3A), the lipid peaks are found at levels comparable to those of the PAHs. By contrast, in the bottom chromatogram (SFE-plus-C_{18}, Figure 3B) the lipids have been largely removed, and PAH peaks are readily dominant.

The quantitative results for PAHs in both SFE methods are given in Table 3. The PAHs in the smoked meat sample were determined by SFE-plus-C_{18} to be in the range of 10.1−25.6 ng/g, with pyrene exhibiting the highest concentration, and benzo[*a*]pyrene detected at the lowest concentration. The PAHs benzofluoranthene, benzo[*e*]pyrene, and perylene were not detected; the detection limit of the instrument was 2.5−4.1 pg, corresponding to 2.5−4.1 ng/g in the original smoked meat sample. In examining the PAH recoveries obtained by the two methods, the conventional SFE method was lower in all cases, yielding calculated recoveries that were 63.1−94.1% of those obtained by SFE-plus-C_{18}. The improvement is most obvious in the region of the chromatogram where the lipid ("c" and "d") background was most severe. In comparing Figures 3A and 3B, the SFE-plus-C_{18} recoveries of fluoranthene and pyrene were raised by 159% and 154%, respectively. In the

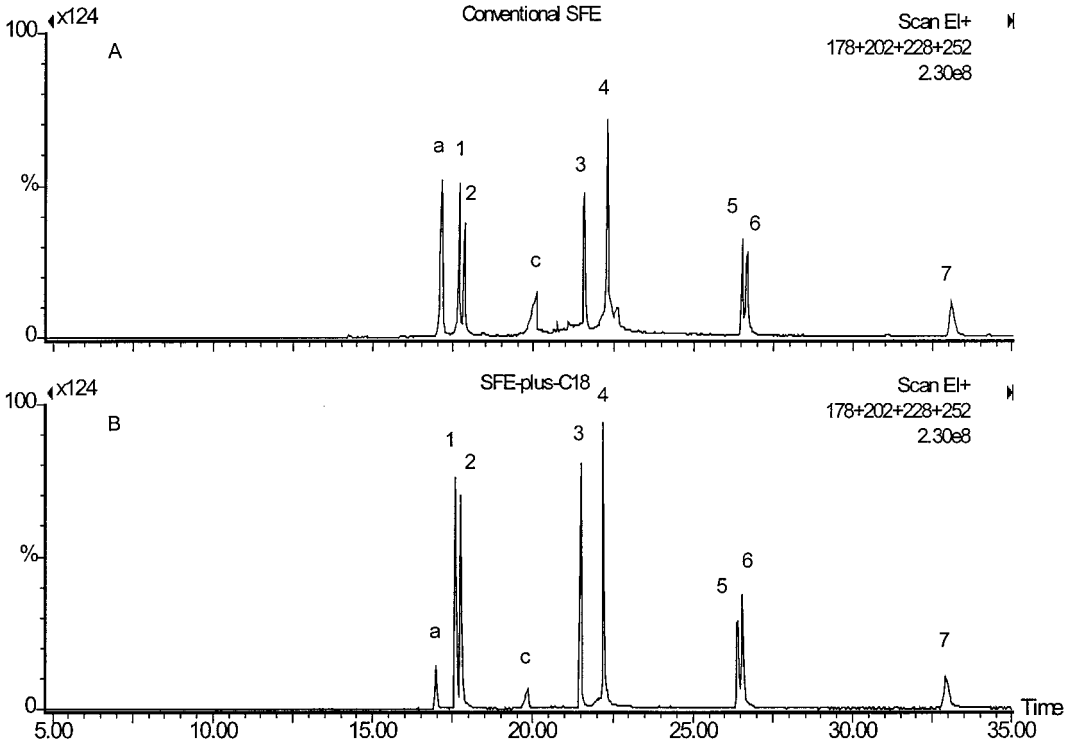

Figure 3. Selected ion chromatograms of *m*/*z* 178, 202, 228, and 252 from extract of pureed smoked meat sample. A: Conventional SFE. B: SFE-plus-C_{18}. Peaks labeled with numbers correspond to PAHs: 1, phenanthrene; 2, anthracene; 3, fluoranthene; 4, pyrene; 5, benzo[*a*]anthracene; 6, chrysene; and 7, benzo[*a*]pyrene. Peaks labeled with letters correspond to EI molecular ions and fragments of lipids "a" and "c", respectively. The full scale *Y*-axis values for Figures 3A and 3B are identical; each has been magnified by a factor of 124 as compared to those in Figure 2.

Table 3. Determination of PAHs in Smoked Meat Samples by SFE Methods

compound	recovery of PAHs in ng/g (mean + SD)		percentage of PAHs lost in conventional SFE vs SFE-plus-C_{18}
	conventional SFE	SFE-plus-C_{18}	
phenanthrene	17.4 ± 1.5	22.1 ± 1.7	21.3
anthracene	14.8 ± 1.8	20.0 ± 1.3	26.0
fluoranthene	12.8 ± 1.9	20.3 ± 1.2	36.9
pyrene	16.6 ± 2.1	25.6 ± 1.3	35.2
benzo[*a*]anthracene	12.4 ± 1.5	16.2 ± 1.0	23.5
chrysene	12.0 ± 2.0	17.3 ± 1.9	30.6
benzofluoranthene	not detected	not detected	
benzo[*e*]pyrene	not detected	not detected	
benzo[*a*]pyrene	9.5 ± 0.9	10.1 ± 1.4	5.9
perylene	not detected	not detected	

chromatogram region where the lipid background was minimal, the recovery of benzo[*a*]pyrene was also raised, but only by 106%. The above results reveal that the higher lipid backgrounds mask and suppress the recovery of the PAHs in the conventional SFE method. In SFE-plus-C_{18}, the lipid background has been largely removed.

To obtain a quantitative estimate of the degree to which the presence of a coeluting lipid can suppress the EI mass spectral signal of an analyte PAH compound, a series of standard solutions was prepared containing a fixed amount of phenanthrene (serving as a model PAH) and increasing amounts of n-tetradecanoic (myristic) acid (a model lipid). The solutions were evaporated and the samples were introduced into the mass spectrometer using the direct insertion (solids) probe. The content of lipid/PAH (μg myristic acid/μg phenanthrene) placed in the six glass sample "cups" was 0:20, 20:20, 100:20, 500:20, 2500:20, and 5000:20. Figure 4 is a plot of PAH peak area (diamonds, corresponding to *Y*-axis scale on left side) vs lipid/PAH weight ratio. As expected, the peak area corresponding to a fixed quantity of PAH (20 μg) decreases with increasing lipid concentration. In fact, the peak area of phenanthrene decreases to less than one-third of its original (lipid-free) value in the presence of 5000 ng of myristic acid. The second plot (triangles) gives the ratio of peak areas of lipid/PAH (corresponding to *Y*-axis scale on right side) vs lipid/PAH weight ratio obtained from the same experiment. Of course, because the quantity of phenanthrene is constant, the lipid/PAH peak area ratio increases with increasing lipid amount.

The two curves in Figure 4 can be used to obtain an approximate correction for the degree of signal suppression present. Assuming that the percentage of signal suppression depends directly upon the relative amounts of PAH and lipid present, one can calculate the lipid/PAH area ratio directly from the EI mass spectrum. This peak area ratio value gives the lipid/PAH weight ratio through the "triangles" plot (Figure 4). In turn, the lipid/PAH weight ratio can provide a PAH peak area value that can be compared to the PAH peak area at zero lipid concentration to indicate the percentage of signal suppression and, hence, the correction factor

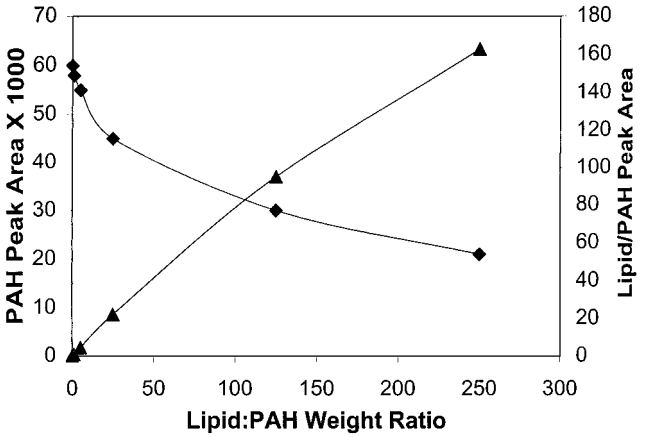

Figure 4. Signal suppression study plotting PAH peak area (left side *Y*-axis, diamonds) and ratio of lipid/PAH peak areas (right side *Y*-axis, triangles) vs lipid/PAH weight ratio (ng/ng) in prepared solid samples of phenanthrene (PAH) and *n*-tetradecanoic acid (lipid). These plots illustrate the progressively increasing level of PAH signal suppression in the presence of rising amounts of lipid.

required. Although no recovery values reported in this paper have been subjected to any correction factor, by using the curves in Figure 4, it can be estimated that the signal for phenanthrene in Figure 2B has been suppressed by approximately 13%.

CONCLUSION

The SFE-plus-C_{18} method, employing addition of nonpolar adsorbent C_{18} (octadecylsiloxane) beads to the SFE chamber, permitted the trapping of interfering lipids, thereby allowing selective extraction and high recoveries of PAHs from lipid-containing matrixes. The SFE-plus-C_{18} method was successfully applied to a high-lipid-containing grocery store food product. The method permitted the efficient and selective extraction of PAHs from the smoked meat sample, and the C_{18} beads largely inhibited the coextraction of interfering indigenous lipids. Because 83−89% of the lipids were adsorbed to the C_{18} during the SFE-plus-C_{18} procedure, the ability to quantify PAHs in the meat sample was substantially improved. Out of 10 targeted PAHs, seven were found, appearing in a range of 10.0−26.0 ng/g from the smoked meat product. Future work will examine further applications of the SFE-plus-C_{18} method for the analysis of PAH compounds in other biological matrixes of high lipid content such as fish.

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