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Determination of 10 Carcinogenic Polycyclic Aromatic Hydrocarbons in Mainstream Cigarette Smoke

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Polycyclic aromatic hydrocarbons (PAHs) are one class of chemical compounds that (1) are present at low to trace levels in unburned cigarette filler, and (2) are predominantly generated during combustion. According to a recent report of the International Agency for Research on Cancer, 10 carcinogenic PAHs together with 53 other known carcinogens are present in cigarette smoke. Accurate quantification of these chemicals helps assess public health risk to both smokers and nonsmokers exposed to second-hand smoke. We have developed and validated a specific and sensitive method for measuring these 10 carcinogenic PAHs in the particulate phase of mainstream tobacco smoke. Cigarette smoke particulate, produced using standard machine smoking protocols, was collected on glass fiber Cambridge filter pads. The particulate matter was solvent extracted, purified by solid-phase extraction, and analyzed by liquid chromatography/atmospheric pressure photoionization tandem mass spectrometry using isotopically labeled analogues as internal standards. Our method's limits of detection ranged from 11 to 166 pg and achieved sufficient reproducibility and accuracy to provide useful information on a range of cigarettes having dramatically different machine-smoked tar and nicotine deliveries. The identity of each PAH analyte was established from chromatographic retention time, analyte-specific fragmentation patterns, and relative peak area ratios of the product/precursor ion pairs. This new method provides higher sensitivity, specificity, and throughput than did earlier methods. We found relatively consistent PAH levels among a selection of domestic full-flavor cigarettes. The PAH levels in smoke from highly ventilated light and ultralight cigarettes were low when smoked using ISO (International Organization for Standardization) conditions. However, if highly ventilated cigarettes were smoked under more intense conditions (e.g., larger or more frequent puffs, vents blocked), their PAH levels equaled or exceeded their full-flavor counterparts under ISO conditions.

KEYWORDS: Polycyclic aromatic hydrocarbons; mainstream cigarette smoke; LC-APPI-MS/MS

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are one class of chemical compounds generated during tobacco smoking (*1*). Hundreds of PAHs and their alkyl derivatives have been identified in cigarette smoke (*2*−*6*). According to a recent publication of the International Agency for Research on Cancer (IARC), 10 carcinogenic PAHs along with 53 other carcinogens are present in cigarette smoke (*7*). These 10 PAHs are benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*j*]-fluoranthene (BjF), benzo[k]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a*,*h*]anthracene (DahA), dibenzo[*a*,*i*]pyrene (DaiP), dibenzo[*a*,*e*]pyrene (DaeP), indeno[1,2,3-*cd*]pyrene (IcdP), and 5-methylchrysene (5MC) (**Figure 1**). PAHs have long been recognized as environmental pollutants. The U.S. Environmental Protection Agency (EPA) has identified 16 priority environmental PAH pollutants including six of those above (*8*, *9*). Although the IARC and EPA lists have some overlap, the IARC 10 PAHs list mainly contains higher molecular weigh PAHs (4−6 rings) that are carcinogenic, whereas the EPA's list of 16 PAHs includes lower molecular weight PAHs (2−5 rings) that are either toxic or carcinogenic.

Methods for detection and quantification of the EPA's list of 16 PAHs are well established (*10*−*12*). Despite slightly different versions, a generic method typically utilizes either gas chromatography (GC) or liquid chromatography (LC) separation followed by ultraviolet (UV), fluorescence, or flame ionization detection (FID). Samples are usually aqueous, though occasionally samples are collected from air or solid waste. Since PAHs were first detected in cigarette smoke (*3*−*5*), researchers have been developing and improving PAH methods in this complex chemical matrix. Unlike an environmental matrix (air, water, soil), the mainstream smoke from cigarettes is a heterogeneous aerosol containing more than 4,000 compounds from multiple

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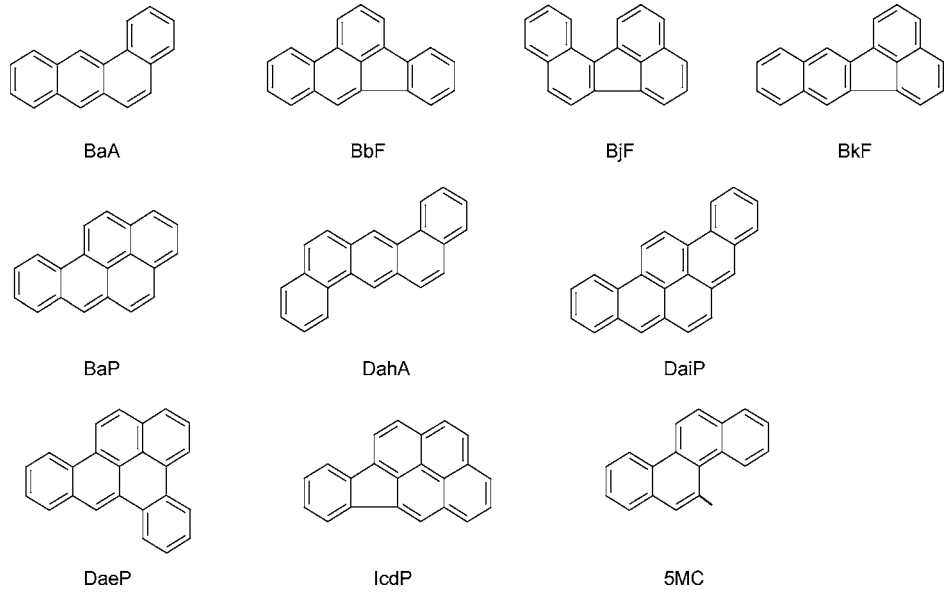

Figure 1. Chemical structures of 10 polycyclic aromatic hydrocarbons.

chemical classes (*13*), and except for a very few lower molecular weight semi-volatile PAHs, most PAHs reside in the total particulate matter (TPM) of cigarette smoke (*14*). Because of the many compounds in TPM, matrix interferences and chemical noise present a major hurdle in achieving good quantification. The simple purification strategies used in preparing environmental samples lack specificity and are not suitable for tobacco smoke samples.

Among the PAHs, benzo[*a*]pyrene has been the most often investigated because of its ability to induce lung tumors (*15*). Studies on PAH levels in cigarette smoke often report only benzo[*a*]pyrene (*16*−*19*). In analytical studies, benzo[*a*]pyrene has often been used as a surrogate for the other PAHs, and the abundant literature concerning it may divert attention from other important PAHs (*2*, *7*, *15*). However, benzo[*a*]pyrene is not the most abundant member from the IARC PAH carcinogen list (e.g., benzo[*a*]anthracene, also an IARC 2A (probably carcinogenic to human) carcinogen, is typically 2−7 times higher in mainstream cigarette smoke than is benzo[*a*]pyrene) (*7*). Therefore, from a public health perspective, understanding the impact of cigarette design and contents on the other PAHs in the list is equally important.

Few studies in the literature have quantitatively reported 10 or more PAHs in mainstream cigarette smoke (*20*−*23*). The method developed by Grimmer and Naujack to analyze 16 PAHs in sidestream smoke and indoor air was the first one in this area (*23*). But that method was cumbersome, with multiple cleanup steps, including numerous extractions and column purifications. Such an approach is too labor intensive for general use in high throughput applications. Furthermore, this method used a single analyte as a surrogate internal standard for the other PAHs and flame-ionization detection resulting in limited sensitivity and specificity (*23*). Two similar methods with streamlined purification and detection strategies were developed with a simplified clean up procedure (i.e., solid-phase extraction or distillation extraction), followed by GC-MS detection (*20*, *22*). With several corresponding deuterium analogues as internal standards, PAH quantification was improved. A recent report also reduced the number of cigarettes per Cambridge pad from 20 to three making it possible to rapidly survey multiple brands of cigarettes (*21*). Still, none of these methods expanded the scope of the 16 EPA's priority PAHs. Little or no recent data are currently available on modern cigarettes for several PAHs on the IARC list, (e.g., benzo[*j*]fluoranthene, dibenzo[*a*,*i*]pyrene, dibenzo[*a*,*e*]pyrene, and 5-methylchrysene). These compounds have higher molecular weights and lower abundances in cigarette smoke, requiring higher sensitivity and specificity, compared with the compounds on the EPA list.

We have developed a method to quantitatively measure all 10 of the IARC PAHs. Our method uses solid-phase extraction (SPE) to concentrate PAHs from mainstream cigarette smoke particulate extracts followed by high performance liquid chromatography/atmospheric pressure photoionization tandem mass spectrometry (HPLC/APPI-MS/MS). The method only requires smoking one cigarette per pad. To improve statistical sampling, the reported values are an average derived from 10 independent measurements. The SPE cleanup can accommodate high throughput automation. Photoionization greatly increases mass spectral sensitivity for all PAHs on the IARC list. We used this methodology to examine and characterize research cigarettes and commercial cigarettes from the major domestic tobacco companies. This is the first comprehensive effort in the last 30 years to report on the levels of several selected carcinogenic PAHs measured in mainstream smoke from modern cigarette brands.

MATERIALS AND METHODS

Reagents, Standards, and Materials. The PAH standards, BaA, BbF, BjF, BkF, BaP, DahA, DaiP, DaeP, IcdP, 5MC, and their isotopically labeled analogues, BaA-$^{13}C_6$, BbF-$^{13}C_6$, BkF-$^{13}C_6$, BaP-$^{13}C_4$, DahA-$^{13}C_6$, DaiP-$^{13}C_{12}$, DaeP-$^{13}C_6$, IcdP-$^{13}C_6$, 5MC-D_3,were purchased from Cambridge Isotope Laboratories (Andover, MA). Acetonitrile, acetone, and cyclohexane were obtained from Sigma (St. Louis, MO) and were HPLC grade. Cambridge filter pads (CFPs, 44 mm glass fiber filter pad) were obtained from Whatman (Maidstone, UK). Reference cigarettes (2R4F) were from the University of Kentucky (Lexington, KY). Commercial cigarettes were purchased from various retail sources in Atlanta, GA.

PAH Standard Solution. A standard solution containing all 10 PAHs was prepared in acetonitrile. According to their relative abundance in cigarette smoke, the concentrations varied from 100 ng/mL to 5 μg/mL. An internal standard solution, containing nine isotopically labeled PAH analogues, was prepared in acetone (1 μg/mL each). For the calibration curves, a set of seven blank CFPs were spiked with various amounts of PAH standard solution and 25 μL of the internal standard solution. Each CFP was then subjected to the same preparation procedure used for smoke samples.

Table 1. Multiple Reaction Monitoring Analysis of 10 PAHs and Their Internal Standards (other parameters (V): declustering potential (*60*); entrance potential (*8*); collision exit potential (*15*))

analyte	precursor ion	product ion	CE[a]	internal standard	precursor ion	product ion	CE[a]
BaA	229.2	228.2[b]	40	BaA-$^{13}C_6$	235.2	234.2	40
		227.2[c]	55				
5MC	242.2	239.2[b]	57	5MC-D_3	245.2	244.2	33
		241.2[c]	32				
BjF	252.2	250.2[b]	60	BbF-$^{13}C_6$	258.2	256.2	60
		248.2[c]	90				
BbF	252.2	250.2[b]	60	BbF-$^{13}C_6$	258.2	256.2	60
		248.2[c]	90				
BkF	252.2	250.2[b]	60	BkF-$^{13}C_6$	258.2	256.2	60
		248.2[c]	90				
BaP	253.2	252.2[b]	40	BaP-$^{13}C_4$	257.2	256.2	40
		251.2[c]	62				
IcdP	276.2	274.2[b]	70	IcdP-$^{13}C_6$	283.2	282.2	50
		272.2[c]	100				
DahA	279.2	278.2[b]	45	DahA-$^{13}C_6$	284.2	283.2	60
		277.2[c]	60				
DaeP	303.2	302.2[b]	45	DaeP-$^{13}C_6$	309.2	308.2	45
		301.2[c]	65				
DaiP	303.2	302.2[b]	45	DaiP-$^{13}C_{12}$	315.2	314.2	45
		301.2[c]	65				

[a] CE (V), collision energy. [b] Quantitation ion. [c] Confirmation ion.

Smoke Particulate Matter Collection. Before smoking, the cigarettes and CFPs were conditioned at 22 °C and 60% relative humidity for at least 24 h. Mainstream smoke TPM generated under ISO smoking conditions (60 s puff interval, 2 s puff duration, and 35 mL puff volume) was collected on individual CFPs using a Cerulean (Milton Keynes, UK) ASM500 16-port smoking machine. The cigarettes were smoked to a butt length of 23 mm or the length of the filter overwrap plus 3 mm, whichever was longer. One cigarette was smoked per pad for each individual sample. Cigarettes from 10 different packs of each brand were smoked to obtain an average smoke particulate level for each PAH. During each smoking run, 2R4F cigarettes were smoked as quality control (QC) samples. After a group of cigarettes were smoked, each CFP was spiked with 25 μL of internal standard solution and processed through the sample preparation scheme.

Sample Preparation. After smoking, the TPM collected on each CFP was extracted using 12 mL of cyclohexane while at the same time shaken at 200 rpm on an orbital shaker (Lab-Line) for 1 h in a sealed 13-mL amber vial. The cyclohexane extracts were loaded on 500-mg Waters Sep-Pak Vac RC silica cartridges (Milford, MA). The column was washed with 6 mL of cyclohexane. Eluents collected from both the load and wash were then dried using a Zymark Turbovap LV evaporator (Hopkinton, MA) and reconstituted in 100 μL of acetonitrile. For analysis, a 10-μL aliquot of this solution was injected onto the HPLC.

LC-MS/MS Analysis. All samples were analyzed using an Agilent 1100 liquid chromatograph (Agilent Technologies, Wilmington, DE) coupled with a PhotoSpray API 4000 triple quadruple mass spectrometer (Applied Biosystems, Foster City, CA). The reconstituted smoke-extract solutions were injected on the HPLC/APPI-MS/MS system twice, on different columns, in a standard dual-column configuration. A Thermo Hypersil Green PAH column (2.1 × 100 mm i.d. 3 μm particle size, Thermo Electron Corporation, Waltham, MA) separated six of the PAHs (BaA, 5MC, BjF, BbF, BkF, and BaP). A Waters Xterra C_{18} MS column (2.1 × 150 mm i.d. 3.5 μm particle size, Waters Corporation, Milford, MA) separated the remaining four PAHs (DahA, IcdP, DaeP, and DaiP). Both columns were equilibrated and run with 100% acetonitrile. The flow rate for the Hypersil column was 250 μL/min, and the run time was 7 min. The flow rate for the Xterra column was 200 μL/min, and the run time was 6 min.

Photoionization of PAHs was achieved using the source operated in positive ion mode. A photodopant, toluene, was infused at a flow rate of 150 μL/min to improve the ionization efficiency. The instrument settings were as follows: curtain gas (N_2) at 40 psi; ion source (N_2): nebulizer gas (gas 1) and lamp gas (gas 2) were at 60 and 20 psi, respectively; source temperature at 400 °C; ion transfer voltage at 800 V; collision gas (N_2) at 4 when vacuum gauge pressure at 2.9×10^{-5} Torr. Mass spectral data on precursor and product ions were collected in multiple reaction monitoring (MRM) mode. The declustering potential, entrance potential, collision energy, and cell exit potential were optimized for each analyte (**Table 1**). Except BjF, specific C-13 labeled internal standards were used for all analytes (**Table 1**). For BjF quantification, BbF-$^{13}C_6$ was used as the internal standard.

Data Analysis. Peak area determinations for all samples, blanks, standards, and QC materials were processed using the Analyst software version 1.4.1 (Applied Biosystems, Foster City, CA). Each ion of interest in the chromatogram was automatically selected and integrated. The peak integrations were manually inspected for errors and if necessary, reintegrated. For each precursor ion, two product fragmentation ions, a quantification ion and a confirmation ion, were collected to improve specificity.

Aggressive Smoking Study. Filter ventilation holes of selected cigarette brands were sealed using short strips of transparent tape (3M, St. Paul, MN). Modified cigarettes were smoked under more intensive conditions (30 s puff interval, 2 s puff duration, and 55 mL puff volume) commonly referred to as the Canadian Intense regimen. TPM collected was then subjected to the identical sample preparation and HPLC/APPI-MS/MS analysis as for regular cigarettes.

RESULTS AND DISCUSSION

Method Development. We examined a variety of solvents (i.e., methanol, methylene chloride, acetonitrile, and acetone) to optimize PAH extraction from CFPs, combined with reverse-phase SPE. This approach had limited applicability for our purpose because these 10 carcinogenic PAHs were in low abundance and are nonpolar. When typical solvents were used for extraction, many interferences appeared in the chromatogram. Furthermore, reverse-phase chromatography required starting with a polar solvent such as a methanol-water mixture and then changing to a less polar solvent. Most of the PAHs examined have very limited solubility. Therefore, because nonpolar solvents were required, hexane and cyclohexane were evaluated. To compare extraction efficiencies, an internal standard mix was added to each sample after cleanup but before injection on column. Both hexane and cyclohexane extraction showed higher sensitivity compared to other polar solvents, and cyclohexane was the better of the two. Cyclohexane extraction was optimized using a series of 2R4F cigarette TPMs extracted

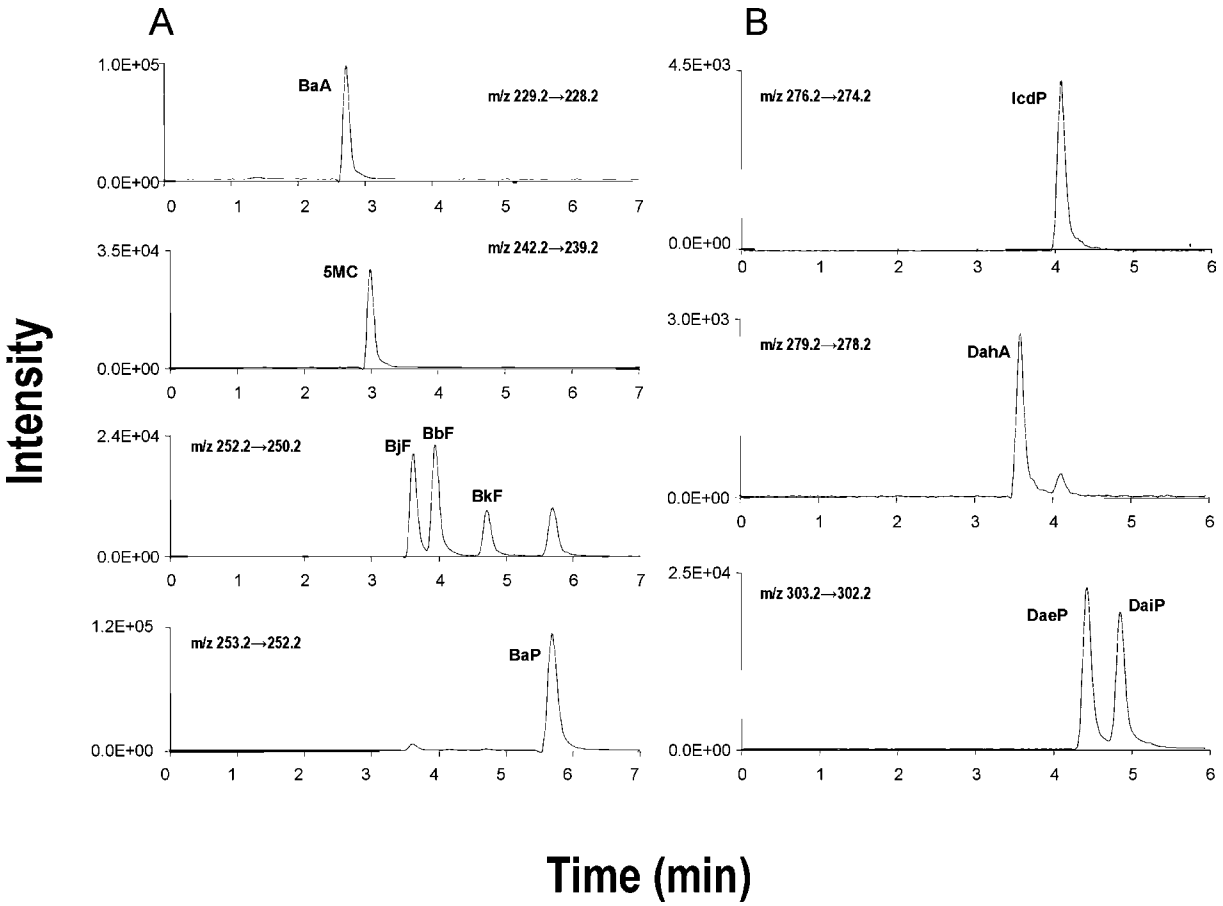

Figure 2. Multiple reaction monitoring chromatograms of PAHs from standard. A. Analytes separated by Hypersil column. BaA, benz[*a*]anthracene (*m*/*z* 229.2 → 228.2); 5MC, 5-methylchrysene (*m*/*z* 242.2 → 241.2); BjF, benzo[*j*]fluoranthene (*m*/*z* 252.2 → 250.2); BbF, benzo[*b*]fluoranthene (*m*/*z* 252.2 → 250.2); BkF, benzo[*k*]fluoranthene (*m*/*z* 252.2 → 250.2); BaP, benzo[*a*]pyrene (*m*/*z* 253.2 → 252.2). B. Analytes separated by Xterra column. IcdP, indeno[1,2,3-*cd*]pyrene (*m*/*z* 276.2 → 274.2); DahA, dibenz[*a,h*]anthracene (*m*/*z* 279.2 → 278.2); DaeP, dibenzo[*a,e*]pyrene (*m*/*z* 303.2 → 302.2); DaiP, dibenzo[*a,i*]pyrene (*m*/*z* 303.2 → 302.2).

in cyclohexane for 0.5, 0.75, 1, 1.5, and 2 h, respectively. The time-dependent study showed that most PAHs reached their maximum recovery after 1 h. We also performed different volumes and multiple extraction studies. In terms of extraction efficiency and solvent consumption, an extraction volume of 12 mL was found to be sufficient.

For the analysis of benzo[*a*]pyrene, Dumont et al. had previously used NH_2 Sep-Pak cartridge for normal phase SPE (*16*). In addition to the NH_2 cartridge, we also evaluated regular silica cartridges and different column capacities for the SPE cleanup procedure. We found that for our method a 500-mg silica cartridge was optimal for all 10 PAHs in terms of recovery and removal of interference.

For our HPLC column selection, we evaluated several commercial columns designed for measuring PAHs in environmental samples, including Waters PAH C_{18} (Waters Corporation, Milford, MA), Thermo Hypersil Green PAH, and Grace Vydac PAH columns (Grace Vydac, Hesperia, CA). The Thermo Hypersil Green PAH column gave the best separation for all 10 PAHs. Compared with the other analytes, however, the sensitivities of four PAHs (DahA, IcdP, DaeP, and DaiP) were much lower. A 30-minute run time was necessary for the last analyte (DaiP) to elute, although most analytes eluted before 15 min. We also evaluated other types of C_{18} columns including Waters Xterra C_{18} MS, Xterra phenyl (Waters Corporation, Milford, MA), and Chromolith SpeedROD (Merck KGaA, Darmstadt, Germany). Although use of the Xterra C_{18} MS column provided very good sensitivity on all 10 PAHs and kept the run time below 10 min, it failed to separate three isomers (BjF, BbF, and BkF). After carefully evaluating the various columns' properties, we decided to separate and analyze six PAHs (BaA, 5MC, BjF, BbF, BkF, and BaP on the Thermo Hypersil Green PAH column and to use the Xterra C_{18} MS column to separate and analyze DahA, IcdP, DaeP, and DaiP (**Figure 2**). By injecting each sample twice using the dual-column configuration, the total run time was 13 min. This approach was much faster than using the Thermo Hypersil column alone, and it maintained the good sensitivity achieved by the Xterra column.

Previous work with PAHs that utilized LC for separation normally used UV or fluorescence detection (*10*, *12*). Because higher molecular weight PAHs are thermally labile and generally have low ionization efficiencies with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), LC/MS detection efficiencies was limited in its ability to identify and quantitate high molecular weight PAHs (*24*). We investigated using standard ESI or APCI to detect these PAHs on a Sciex API 4000 triple quadruple mass spectrometer. The ESI source failed to ionize PAHs effectively, and, except for BaA and BaP, APCI had poor ionization efficiencies. This is likely why most previous mass spectrometric methods have focused on the lower molecular weight PAHs that are more amenable to analysis mainly by GC techniques (*4*, *5*, *14*, *20*, *21*, *22*). In 2000, Robb, et al. introduced a dopant-assisted APPI technique, which is easily compatible with LC and achieved dramatically improved ionization of nonpolar compounds such as PAHs (*25*).

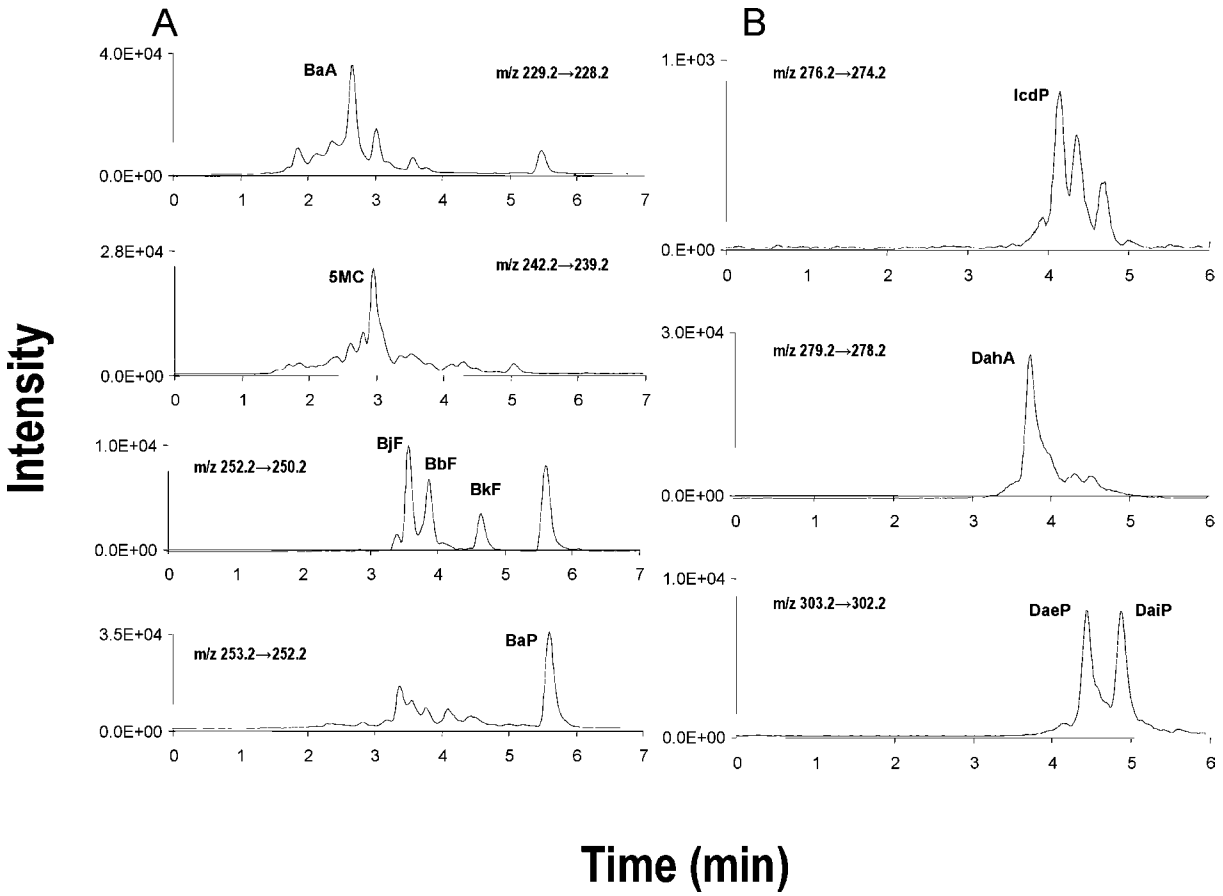

Figure 3. Multiple reaction monitoring chromatograms of PAHs from 2R4F. A. Analytes separated by Hypersil column. B. Analytes separated by Xterra column. Analyte abbreviations and transition states are the same as in **Figure 2**.

Recently, a study on environmental PAH samples by LC/MS using a photospray prototype showed promising results (*26*). During the ionization process, the UV lamp photoexcites infused toluene used as dopant, ultimately resulting in improved ionization of selected classes of analytes. The photoexcited toluene acts as a reactive intermediate, transferring energy and charge to PAH molecules ($M^{\bullet+}$). Often, PAH molecules acquire a proton during the ionization process and form a protonated molecule ($[M + H]^+$) (*24*). For each PAH, both molecular ions and protonated molecules form during the complex photoionization process; however, these two ions have compound-specific differences in their relative abundances that influence relative sensitivity. Either the molecular ion or protonated molecules were selected for quantification on the basis of sensitivity and specificity criteria (**Table 1**).

Chromatography. Reconstructed ion chromatograms for the PAHs extracted from the mainstream smoke particulate matter show the resolution achieved in actual smoke samples (**Figure 3**). Because of interference in the complex smoke matrix, the extracted ion chromatograms exhibited higher chemical backgrounds compared to standard solutions (**Figure 2**). Most PAHs exhibited good sensitivity and chromatographic resolution except for 5MC and DahA (**Figure 3**). During the combustion of a cigarette, hundreds of structurally similar PAHs are generated. Many have structural isomers of equal molecular weight and overlapping retention times, potentially complicating detection. By optimizing the chromatography and appropriate precursor/product ion selections, we separated many of the PAH isomers present in mainstream smoke such as BaA (precursor ion having *m*/*z* 229.2 to product ion having *m*/*z* 228.2) and chrysene (228.2 → 226.2), three benzofluoranthene isomers (252.2 → 250.2), benzo[*a*]pyrene (253.2 → 252.2), IcdP (276.2 → 274.2), and benzo[*ghi*]perylene (277.2 → 276.2). However, 5MC and DahA were difficult to cleanly separate from their isomers having similar fragmentation patterns. For those peaks that were not properly integrated by software, manual integrations were performed. Generally, to achieve reproducible results, integration was accomplished by drawing a tangent line from valley to valley.

Method Validation. Extracts prepared from a blank CFP were routinely analyzed for any coeluting interferences or sample carry-over from previous PAH-containing samples. No false positive responses were observed for any of the PAH analytes in the blank samples. Measurements also were made to determine how much native analyte contributed to the isotope-labeled internal standard and vice versa. No significant cross interference was observed.

The detection limit (LOD) for each PAH was estimated from calibration curves as three times the standard deviations extrapolated to zero concentration. The estimated method LODs for all 10 PAHs were in the pg range. Since it is not possible to obtain PAHs-free smoke particulate matrix, we used standard mixtures spiked on the pad. The analytical LODs are expected to be slightly higher than the method LODs. Given the estimated PAHs delivered in mainstream smoke to be in ng range per cigarette, our method has more than sufficient sensitivity for detecting PAHs in mainstream cigarette smoke (**Table 2**).

The overall method recovery of PAHs from cigarette smoke was calculated as RFa/RFb, where RFa and RFb are the PAH response factors obtained from spiking the smoke sample with the isotopically labeled standard after and before the extraction process, respectively. For most PAHs, recovery rates are

Table 2. Method Validation Parameters for Measuring Polycyclic Aromatic Hydrocarbons in Tobacco Smoke Particulate ($n = 5$)

analyte	standard range (ng)	nominal concn (ng)	mean accuracy (%)	precision (%)	method recovery (%)	LOD[a] (pg)
BaA	2–200	10	102	0.9	77	166
		40	99	3.9		
5MC	1–100	5	104	10.5	47	94
		20	104	11.5		
BjF	2–200	5	101	2.2	34	100
		20	163	15.7		
BbF	2–200	5	102	0.7	46	89
		20	102	10.6		
BkF	1–100	2.5	93	4.2	45	34
		10	101	2.9		
BaP	2–200	5	93	2.7	63	67
		20	100	2.8		
IcdP	1–100	2.5	98	1.5	63	18
		10	83	10.2		
DahA	1–100	2.5	89	6.7	57	22
		10	108	11.6		
DaeP	0.2–20	0.5	95	1.2	29	11
		2	107	1.4		
DaiP	0.2–20	0.5	97	4.7	29	23
		2	103	4.5		

[a] LODs were obtained by spiking standards on clean pads since it was impossible to acquire PAHs-free smoke particulate matrix.

Table 3. Polycyclic Aromatic Hydrocarbon Levels (ng per cigarette) in Domestic United States Cigarettes ($n = 10$)[a]

analyte (ng/cig)	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F	Brand G	Brand H	Brand I	2R4F	IARC range[b]
BaA	45.2	50.0	61.1	50.0	43.2	38.2	47.0	39.7	66.6	33.5	20–70
5MC	2.5	2.8	3.3	3.0	2.7	2.8	3.7	2.5	3.9	2.7	ND–0.6
BjF	18.5	17.5	24.3	22.7	22.8	17.5	23.4	14.3	23.3	10.4	6–21
BbF	10.3	9.3	12.1	11.7	11.4	11.5	9.7	5.1	11.4	5.9	4–22
BkF	4.1	3.3	4.5	4.5	3.3	3.6	3.7	2.5	5.0	2.2	6–12
BaP	13.5	12.3	15.6	15.5	13.3	11.8	13.8	10.0	15.8	8.8	8.5–11. 6
IcdP	9.3	7.3	10.0	11.2	10.2	9.1	10.3	7.9	10.3	5.9	4–20
DahA	4.8	5.9	6.2	6.2	4.1	4.7	4.1	3.6	4.4	4.7	4
DaeP	2.4	2.1	2.6	2.0	2.3	2.2	2.4	1.9	2.6	1.5	present
DaiP	1.1	1.0	1.2	1.0	1.0	0.9	1.0	0.9	1.1	0.7	1.7–3.2

[a] Smoking regimen: ISO conditions (35 mL puff volume, 60 s puff interval). [b] Reference 7.

moderate except for DaeP and DaiP (**Table 2**). This could be partially attributed to the partitioning phenomenon between surfaces and solvents during extraction. To achieve the accuracy reported here, these data confirm the necessity of labeled internal standards.

The accuracy of the method was assessed by spiking known amounts of the PAHs on CFPs containing TPM collected from 2R4F cigarettes. Analytes were spiked at two concentrations: one at half the amount in 2R4F cigarette smoke particulate matter, and the other at double the amount in 2R4F. Accuracy was calculated as the mean of the experimentally determined concentration from replicate analysis divided by the nominal concentration. The precision of the method was determined by calculating the relative standard deviations (RSD) of five replicate measurements. The mean accuracies for all analytes ranged from 83% to 108% except for BjF at the high spiking level (163%). Precisions for all analytes were less than 12% except for BjF at the high spiking level (15.7%) (**Table 2**). Labeled BbF does not appear to be completely effective in characterizing the effect of chemical interference on BjF in the smoke-imbedded CFP. We expect the accuracy of BjF would improve if an isotopically labeled analogue had been available for use as an internal standard.

PAH Levels in Mainstream Smoke from Domestic Cigarettes. Some of PAH levels in cigarette smoke referenced in the IARC monograph were obtained more than 30 years ago (*7*). In addition to research cigarettes, we ran a survey of representative American blended cigarettes. Literature and previous studies indicated filter ventilation is a key parameter influencing smoke delivery (*21*, *27*, *28*). To minimize any artifacts associated with air dilution of mainstream smoke in cigarette varieties with high levels of filter ventilation, we mainly focused on full-flavor king-size cigarette brand variants. Using available market share data, we selected nine cigarette brands from the leading domestic U.S. tobacco companies (Brands A, B, C, D, E, F, G, H, and I). Brands G, H, and I are mentholated. Levels of PAHs in smoke particulate from each brand were measured and compared with research cigarette as well as data referenced in the IARC monograph (**Table 3**). In general, among the nine brands of cigarettes, levels of PAHs were relatively consistent. We observed no statistically significant differences between regular and menthol full-flavor cigarettes. Our findings were consistent with the reference data listed by IARC (*7*) except 5MC, BkF, and BaP. Deliveries of 5MC and BaP in cigarette smoke were higher than IARC reference data, (*7*) whereas BkF levels determined in this study were lower. In the IARC report (*7*), DaeP was only listed as being present; but in these 10 brands we observed a range of 1.9 to 2.6 ng per cigarette. Compared to the result from our previous study by GC-MS analysis, the measured amounts for overlapping analytes were consistent (*21*).

The physical design of cigarettes has changed dramatically in the past 30 years (*1*). Many low delivery brands having high filter ventilation (typically labeled light or ultralight variants by the manufacturers) have emerged. Smokers may believe that

Table 4. Polycyclic Aromatic Hydrocarbon Levels (ng per cigarette) in Selected Cigarettes under Different Smoking Regimes[a]

			BaA	5MC	BjF	BbF	BkF	BaP	IcdP	DahA	DaeP	DaiP
Brand B	full	ISO	50.0	2.8	17.5	9.3	3.3	12.3	7.3	5.9	2.1	1.0
		CAN	86.3	5.7	22.2	12.3	5.4	21.8	16.5	7.7	4.1	1.8
	light	ISO	40.9	2.4	9.4	5.2	2.6	11.6	6.5	2.8	1.2	1.0
		CAN	82.9	4.2	22.5	10.7	5.7	19.8	11.5	6.4	2.0	1.8
	ultra	ISO	22.0	1.5	4.7	3.3	1.7	6.5	3.7	2.3	0.8	0.6
		CAN	80.7	4.1	18.5	9.5	5.1	17.4	10.1	4.9	2.3	1.4
Brand E	full	ISO	43.2	2.7	22.8	11.4	3.3	13.3	10.2	4.1	2.3	1.0
		CAN	103.8	6.2	28.5	15.9	7.3	25.9	21.1	7.7	4.7	2.1
	light	ISO	33.2	1.5	7.4	5.0	2.5	10.9	7.1	2.5	1.2	0.8
		CAN	82.6	4.8	27.0	14.6	7.3	23.7	15.9	4.8	2.4	1.7
	ultra	ISO	19.2	1.4	3.9	3.2	1.4	6.0	3.7	1.8	0.6	0.5
		CAN	57.5	5.2	14.6	9.0	4.1	14.6	10.1	4.5	1.5	1.2
Brand I	full	ISO	66.6	3.9	23.3	11.4	5.0	15.8	10.3	4.4	2.6	1.1
		CAN	106.4	6.9	33.3	18.4	7.9	27.5	23.3	7.1	5.2	2.4
	light	ISO	31.9	2.5	6.6	4.3	1.8	8.2	5.8	1.7	0.7	0.6
		CAN	54.6	4.4	20.6	10.8	4.8	14.9	10.9	4.0	1.6	1.0
	ultra	ISO	25.6	2.5	6.4	4.5	2.2	8.3	5.2	1.6	0.7	0.4
		CAN	55.5	5.3	16.9	10.4	5.3	16.2	10.9	4.2	1.5	1.0

[a] ISO: ISO conditions (35 mL puff volume, 60 s puff interval); CAN: intensive conditions (55 mL puff volume, 30 s puff interval, 100% vent holes block). Full ISO data are taken from **Table 3**.

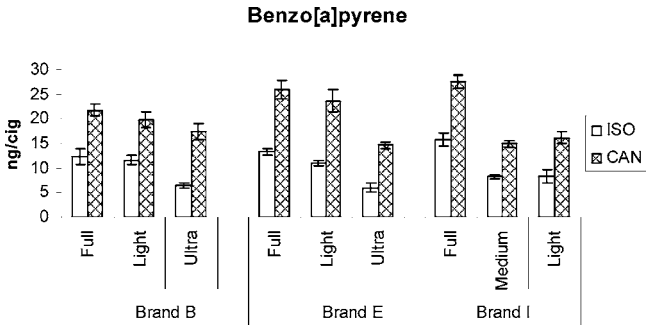

Figure 4. Benzo[*a*]pyrene deliveries in selected full-flavor, light and ultralight cigarettes. ISO: ISO conditions (35 mL puff volume, 60 s puff interval); CAN: intensive conditions (55 mL puff volume, 30 s puff interval, 100% vent hole block).

such products lower their exposure to harmful substances (*29*). Studies have shown, however, that smokers tend to take larger puffs, take more puffs, and/or block vents on high ventilation cigarettes (*28*, *30*, *31*). To test whether smoking behavior changes will affect PAH deliveries in mainstream smoke, we selected several highly ventilated brands (e.g., Brand B light and ultralight variants, Brand E light and ultralight variants, Brand I medium and light variants) and analyzed their PAH deliveries under different smoking conditions. As expected, under standard ISO puffing regimen, these cigarettes have lower levels of PAHs in mainstream smoke compared to their full flavor counterparts, largely due to air dilution through the filter ventilation holes (**Table 4**). But when these cigarettes were smoked using Health Canada's more intensive puffing regimen, including larger puffs (55 mL), shorter intervals (30s) and 100% filter vent holes blockage, the deliveries of PAHs of these light and ultralight cigarettes equaled or exceeded full-flavor cigarettes the mainstream deliveries obtained under the ISO regimen (**Table 4**). For example, benzo[*a*]pyrene deliveries in selected light and ultralight cigarettes smoked using the intense regimen typically were twice that of the ISO smoking regimen (**Figure 4**). Our findings suggest that if smokers of high ventilation cigarettes compensate their smoking behavior by blocking vent holes or by taking larger or more frequent puffs, they will be exposed to higher amounts of PAHs than predicted using the ISO smoking regimen, regardless of whether they smoke full-flavor, light, or ultralight varieties of a particular brand. In addition to the PAHs, smokers are exposed to many other harmful compounds (*32*). At present, the only proven means to reduce the risk associated with smoking is cessation.

In summary, our new HPLC/APPI/MS/MS method allows rapid determination of 10 PAHs from mainstream cigarette smoke that previously had been difficult to quantitatively analyze. The combined HPLC and photoionization tandem mass spectrometry offers several inherent advantages over the UV or fluorescence method for PAH quantitation in terms of unambiguous identification for these PAHs and improved sensitivity. The data from the ISO and the more intense Canadian smoking regimens demonstrate the utility of the method by producing a wide range of smoking deliveries that likely span most human intake ranges. In addition to excellent reproducibility and accuracy through the use of isotope-labeled analogues as internal standards, the ease of sample preparation accommodates high throughput automation. Among these 10 PAHs, only BaP has been extensively studied in mainstream tobacco smoke. There are very few reports on the remaining nine PAHs. Data on 5MC, BjF, DaeP, and DaiP in most review articles including IARC were obtained more than 30 years ago (*7*, *33*, *34*). Our findings provide updated mainstream smoke deliveries for these analytes in modern cigarette products.

SAFETY

Personnel involved in weighing, diluting, or otherwise manipulating the compounds used were instructed in the safe handling of chemicals. These instructions included the wearing of personal protection items and proper laboratory practices. All compounds were handled in a fume hood, and personnel used appropriate protective safety glasses, gloves, and lab coats.

ABBREVIATIONS USED

PAHs, polycyclic aromatic hydrocarbons; ISO, International Organization for Standardization; IARC, International Agency for Research on Cancer; BaA, benz[*a*]anthracene; BbF, benzo[*b*]fluoranthene; BjF, benzo[*j*]fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[*a*]pyrene; DahA, dibenz[*a*,*h*]anthracene; DaiP, dibenzo[*a*,*i*]pyrene; DaeP, dibenzo[*a*,*e*]pyrene; IcdP, indeno[1,2,3-*cd*]pyrene; 5MC, 5-methylchrysene; EPA, U.S. Environmental Protection Agency; TPM, total particulate matter; GC, gas chromatography; LC, liquid chromatography;

UV, ultraviolet; SPE, solid-phase extraction; HPLC/APPI-MS/MS, high performance liquid chromatography/atmospheric pressure photoionization tandem mass spectrometry; CFP, Cambridge filter pad; LOD, limit of diction; RSD, relative standard deviation.

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