Analysis of Polycyclic Aromatic Hydrocarbons in the Particulate Phase of Cigarette Smoke Using a Gas Chromatographic–High-Resolution Mass Spectrometric Technique

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Abstract

A new procedure is developed for the extraction of polycyclic aromatic hydrocarbons (PAHs) from the particulate phase of cigarette smoke. The procedure applies solid-phase extraction using a Bond Elut CH cartridge as a sample preparation step. The efficiency of the cleanup procedure is verified using a gas chromatographic (GC)–high-resolution mass spectrometric (MS) technique, proving that no interference occurs in the PAHs' determination. The efficient cleanup allows GC detection using either high- or low-resolution MS detection. Enhanced sensitivity is obtained using GC–MS and selected ion monitoring. This new technique has several advantages over other reported techniques. The method is simple and robust and has good repeatability and accuracy. The estimated detection limit is 0.1 ng/cigarette for benzo[a]pyrene. In addition to that, the recovery from the smoke pad in which the smoke is collected is approximately 97% for all PAHs. Results for the PAH analyses for 1R5F, 1R4F, and 1R3 Kentucky reference cigarettes are reported in this study. These results provide useful evidence for clarifying the controversy about previously reported data.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are present in the combustion products of many organic materials, including tobacco (1). Benzo[a]pyrene (BAP) and other PAHs are included in the list of tobacco smoke components considered to be biologically active (2) and are currently determined in smoke and tobacco products. Analytical techniques reported in the literature used to quantitate PAHs in particulate-phase smoke (3–11) include procedures for sample preparation such as extractions, solid-phase extraction (SPE), simultaneous distillation and extraction (SDE), and analytical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) with mass spectrometric (MS) detection. Some of the reported techniques analyze only BAP. Only a few studies (3,4) have been conducted for a series of PAHs. This study describes a newly developed technique for PAH analysis. The procedure uses a new solid phase for sample preparation and achieves sample cleanup with excellent extraction efficiency.

Experimental

The chemicals used in this study were obtained from different vendors. Anthracene-d_{10}, dibenzanthracene-d_{14}, and BAP-d_{12} were purchased from CDN Isotopes (Quebec, Canada). Fluorene-d_{10} and pyrene-d_{10} were purchased from Cambridge Isotope Laboratories (Andover, MA). Naphthalene-d_8 was purchased from ACROS Organics (Morris Plains, NJ). Chrysene, dibenzanthracene, naphthalene, pyrene, perylene, fluorene, anthracene, phenanthrene, and benzanthracene were all purchased from CHEM SERVICE (Chester, PA). The rest of the PAHs were obtained from Aldrich Chem. Co. (Milwaukee, WI). All of the HPLC-grade solvents were purchased from Fisher Scientific Co. (Norcross, GA). A Bond Elut CH-SPE column (1 g, 6 mL) was purchased from Varian (Walnut Creek, CA).

For the analysis of PAHs, the particulate-phase smoke from 20 cigarettes was collected using a Borgwaldt RM 20/CS smoking machine with a 92-mm smoke pad. Smoking can be performed under any specific protocols such as those recommended by the U.S. Federal Trade Commission (FTC) (12) or the International Standard Organization (ISO) (13–15). After smoking, the pad was spiked with an internal standard (IS) solution of 100 µL PAH in dichloromethane and then extracted with 35 mL methanol for 30 min using a mechanical wrist shaker. The pad was removed from the methanol solution after extraction, and the methanol extract was mixed with 65 mL deionized water. The whole sample solution of approximately 100 mL was loaded onto a Bond Elut CH-SPE column under vacuum using an Alltech 12-port vacuum manifold. The SPE column volume used in this procedure was

6 mL and the sorbent mass was 1.0 g. The Bond Elut CH sorbent was conditioned in advance with 10 mL methanol followed by 10 mL water–methanol (65:35, v/v). After loading the sample, the column was washed with water (3 × 10 mL) followed by water–methanol (10 mL) for the cleanup step. After drying the SPE column under nitrogen for 5 min, the PAHs were eluted with 4 mL of cyclohexane to obtain a processed sample for GC–MS analysis. A diagram showing the sequence of sample preparation operations is given in Figure 1.

The analysis of the processed sample was performed on an Agilent 6890/5973 GC–MS system. The GC was equipped with a 30-m-long, 0.25-mm-i.d., 0.25-µm-film-thickness ZB-5 capillary column. The oven was programmed at an initial temperature of 45°C for 7 min, a heating rate of 8.5°C/min to 360°C, and a final time of 5 min. The MS was operated under selected ion monitoring (SIM) mode. Because all PAHs generate the molecular ion as the base peak in their mass spectra, the molecular ions were used for SIM. The selected ions for different PAHs are listed in Table I.

The GC–high-resolution (HR) MS analysis was performed on a Micromass Autospec MS coupled with an Agilent 5890 GC. The GC was equipped with a 30-m-long, 0.25-mm-i.d., 0.25 µm-film-thickness ZB-5 capillary column. The oven was programmed at an initial temperature of 45°C for 7 min, a heating rate of 8.5°C/min to 360°C, and a final time of 5 min. The MS was operated under HR-selected ion recording (SIR) mode. The resolution of the MS was tuned at 5000 (5% valley). The lock peak of the selected mass was from perfluorokerosene eluted separately from a heated reservoir into the ion source.

The quantitative analysis of the PAHs was performed using the peak-area ratio of each PAH and its corresponding deuterated standard. For this purpose, response factors (RF) for each PAH were initially determined. A standard solution was made containing exactly the same amount of each PAH and deuterated PAH. The RFs for a specific PAH was calculated by dividing the areas of the chromatographic peaks of the extracted ion for a PAH ($AREA_{PAH}$) by that of the corresponding deuterated PAH ($AREA_{PAH\text{-}d}$). The RFs for each compound were obtained by averaging the RFs calculated from three runs using the formula:

$$RF = AREA_{PAH} / AREA_{PAH\text{-}d} \qquad \text{Eq. 1}$$

Because not every analyzed PAH had a corresponding deuterated standard, the nearest deuterated PAH in the chromatogram was taken for the determination of the RF. After the GC–MS separation and detection, the peak areas of extracted ions for all of the analytes ($AREA_{analyte}$) and the deuterated ISs added on the pad ($AREA_{IS}$) were measured and the concentration of each component was calculated using the following formula:

$$PAH = (((AREA_{analyte} / AREA_{IS}) / RF) \times IS) / (\text{No. of smoked cigarettes}) \qquad \text{Eq. 2}$$

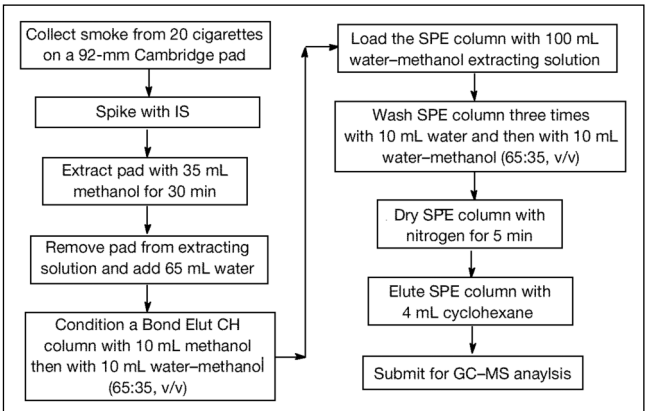

Figure 1. Schematic flow chart of an SPE procedure.

By using a constant amount of deuterated standard it must be shown that the peak-area ratio has a linear dependence on the analyzed PAH concentration for a range that covers all of the possible PAH concentrations in particulate-phase smoke. As an example, a calibration curve for the quantitation of BAP was constructed. BAP was chosen for the study because it is the compound most frequently analyzed among the PAHs. For this study, seven smoke pads were spiked separately with 2.5, 5, 25, 60, 150, 400, and 1000 ng of BAP along with 100 ng BAP-d_{12} as an IS. The amount of BAP spiked on the smoke pads was equivalent to 0.125, 0.25, 1.25, 3.0, 7.5, 20, and 50 ng/cigarette, respectively, for the particulate-phase smoke of 20 cigarettes. This range covered the possible BAP concentration not only in mainstream smoke, but also in sidestream smoke for almost all of the cigarettes. The calibration curve for BAP obtained from triplicate measurements is shown in Figure 2. The R^2 value for the calibration curve was 0.9993. A linear concentration range between 0.125 and 50 ng/cigarette was observed for BAP. The highest concentration was 400 times higher than the lowest concentration for this linear range.

The lowest concentration for the calibration curve in Figure 2 was set to be 2.5 ng BAP in final eluate solution, which is equiva-

Table I. The Ions Selected for PAH Monitoring

PAH	Ion	Remark
Naphthalene-d_8	136	IS
Naphthalene	128	analyte
Fluorene	176	analyte
Fluorene-d_{10}	166	IS
Phenanthrene	178	analyte
Anthracene-d_{10}	188	IS
Anthracene	178	analyte
Fluoranthene	202	analyte
Pyrene-d_{10}	212	IS
Pyrene	202	analyte
Benzofluorene	216	analyte
Benzanthracene	228	analyte
Chrysene-d_{12}	240	IS
Chrysene	228	analyte
Benzo[b,k]fluoranthene	252	analyte
BEP	252	analyte
BAP-d_{12}	264	IS
BAP	252	analyte
Perylene	252	analyte
Dibenzanthracene-d_{14}	292	IS
Dibenzanthracene	278	analyte
Benzoperylene	276	analyte

lent to 0.125 ng/cigarette for the smoke of 20 cigarettes. The half value for the lowest concentration was 1.25 ng BAP in solution. Thus, it was conservatively established that the detection limit was 2 ng for the amount of BAP in the eluate solution and 0.1 ng/cigarette for the BAP concentration in the particulate-phase smoke of 20 cigarettes by using this newly developed technique.

Results and Discussion

The analysis of PAHs in mainstream cigarette smoke condensate (MSS) encounters the problem of interferences from the complex matrix of cigarette smoke. In particular, long-chain hydrocarbons present in MSS show a rather similar behavior with PAHs in many separations. These hydrocarbons interfere in the GC–MS analysis of PAHs because they elute from the GC column in a time range close to that of specific PAHs. Also, the mass spectrum of these hydrocarbons contain fragments with nominal *m/z* values equal to that of the base peak in the spectrum of PAHs. For example, a fragment ion at *m/z* 252 would generate a high background in the time range in which BAP elutes and could create interferences to the molecular ion of BAP (also at *m/z* 252). An important measure for a sample cleanup procedure is to eliminate the possible interference of the long-chain hydrocarbons with PAH analysis. Only the application of a GC–HR-MS can positively prove the elimination of the interferences.

The proof of the efficiency for the cleanup procedure using SPE will now be discussed for the case of BAP. Although the molecular ion of BAP and a fragment ion from long-chain hydrocarbons have the same nominal mass of 252, these two ions have different chemical structures and therefore different exact masses. The chemical structure for the molecular ion of BAP is $C_{20}H_{12}^{+}$, leading to an exact mass of 252.0939. The structure for a fragment ion from long-chain hydrocarbons at *m/z* 252 is $C_{18}H_{36}^{+}$, which has the exact mass of 252.2817. The mass difference between these two ions is 0.1878. In order to separate these two ions by an MS, a mass resolution higher than 1500 (at 50% valley) is needed. The conventional quadrupole or ion-trap MSs that

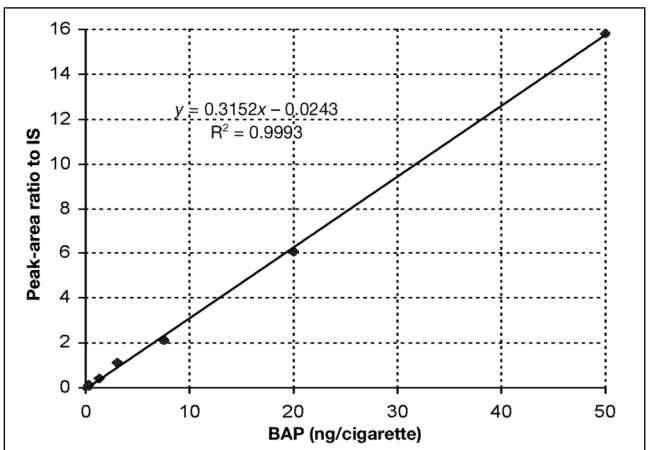

Figure 2. Calibration curve for BAP determination.

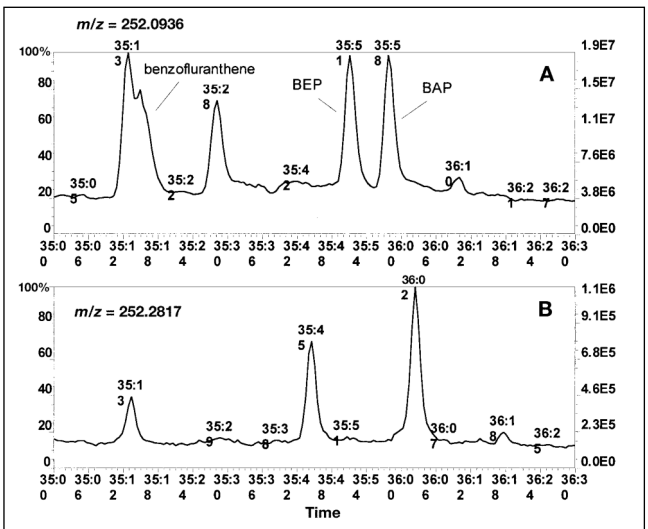

Figure 3. Single ion chromatograms for the MSS of a 1R4F Kentucky reference cigarette showing (A) PAHs and (B) hydrocarbons using HR-MS detection.

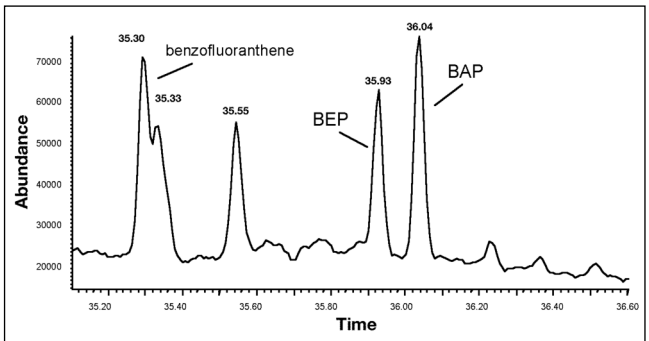

Figure 4. Single ion chromatograms for the MSS of a 1R4F Kentucky reference cigarette showing PAHs in LR-MS detection

Table II. Comparison of the Results of the Analysis of PAHs from the MSS of a 1R4F Kentucky Reference Cigarette Obtained in HR- and LR-MS Detection

	HR		LR	
	Average	Standard deviation	Average	Standard deviation
Naphthalene	292.8	7.2	311.8	6.2
Fluorene	144.5	4.8	129.0	2.2
Phenanthrene	76.3	6.0	68.1	4.0
Anthracene	34.5	2.9	36.0	2.3
Fluoranthene	42.6	1.4	45.9	0.2
Pyrene	27.9	1.3	26.9	0.5
Benzofluorene	20.7	0.6	22.7	1.1
Benzanthracene	10.5	0.4	10.1	0.1
Chrysene	14.0	0.5	13.5	0.1
Benzofluoranthene	7.3	0.5	6.6	0.1
BEP	4.0	0.2	3.5	0.1
BAP	4.6	0.2	4.7	0.0
Perylene	0.9	0.1	0.6	0.1
Dibenzanthracene	0.4	0.1	0.2	0.0
Benzoperylene	1.0	0.1	0.9	0.1
Total PAH (ng)	**682.0**			**675.4**
TPM (mg)	**10.9**		**10.9**	
PAH per TPM (ng/mg)	**62.4**		**61.8**	

operate under a unit mass resolution are unable to separate these two ions. Only an HR-MS can perform this mass separation.

The result of an HR-mass separation of *m/z* 252.0939 and *m/z* 252.2817 for a smoke sample processed as previously described is shown in Figure 3. As seen in this figure, there was no noticeable interference from *m/z* 252.2817 to the molecular ion of BAP at *m/z* 252.0939. As seen in Figure 3, the interference between BAP and the long aliphatic hydrocarbon was minimal. Also, the hydrocarbons were at much lower concentration levels compared with BAP or benzo[e]pyrene (BEP), which allows for a low-resolution (LR) measurement of these compounds. An example of a single ion chromatogram for the MSS of a 1R4F Kentucky reference cigarette showing PAHs performed with LR-MS detection is given in Figure 4. As shown in Figure 4, very good peak shape and separation can be obtained even using LR-MS detection. The quantitative result of a BAP level in the particulate-phase smoke of 1R4F cigarettes obtained from a magnetic sector (HR) MS was identical to that obtained from a quadrupole (LR) MS. The results are given in Table II. It can be concluded from the HR-MS experiment that the newly developed SPE sample cleanup procedure eliminates the possible interference of a fragment ion of long-chain hydrocarbons to the quantitative analysis of BAP. Thus, an LR-GC–MS system can be used for the analysis of PAHs at relatively low cost and short turnaround time.

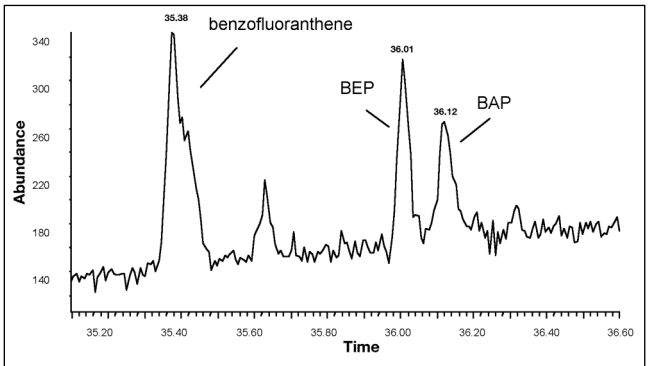

Figure 5. Evaluation of a detection limit showing an extracted ion chromatogram for a sample containing the equivalent of 0.1 ng/cigarette BAP.

The detection limit for BAP analysis has been tested for a real sample. 1R5F Kentucky reference cigarettes have been chosen for this test. The reported data for the BAP concentration of 1R5F in particulate-phase smoke was in the range of 1.1 to 1.5 ng/cigarette. The test was conducted to collect the particulate-phase smoke of 5 cigarettes, and only one-third of the extracts was loaded on the SPE column for extraction and cleanup. Thus, the amount of BAP in the final elute solution was approximately 2 ng. Converting this amount of BAP into the particulate-phase smoke of 20 cigarettes is approximately 0.1 ng/cigarette. The results were obtained performing three replicates for each run.

The result for the detection limit test is demonstrated in Figure 5, which shows an extracted ion chromatogram of *m/z* 252. Benzo[b,k]fluoranthene, BEP, and BAP (which have the same molecular ions at *m/z* 252) were all present in the chromatogram. The signal-to-noise (S/N) ratio for the BAP peak was above 5, which was more than the required S/N ratio for the detection limit. The noise level was measured for an area of the chromatogram next to the BAP peak in which no other peaks were present. A blank smoke sample with no BAP was not available.

In order to verify the repeatability of this newly developed SPE technique, the determination of PAHs in particulate-phase smoke was conducted for 1R5F, 1R4F, and 1R3 Kentucky reference cigarettes, representing cigarettes with different total particulate matter (TPM) levels. The cigarettes were smoked under FTC conditions. Each reference was run four times. The four replicates were smoked and analyzed randomly with other samples within a time period of two weeks. The data reported in Table III were taken as averages. The results showed an excellent repeatability. All of the PAHs except for dibenzanthracene had a standard deviation less than 10%. The reason that dibenzanthracene had a relatively high deviation was because of its very low concentration. The average standard deviation for the fifteen PAHs measured from three different cigarettes was 6.2%.

Table III. Levels of PAHs in Kentucky Reference Cigarettes

	1R5F		1R4F		1R3	
PAH	Average (ng/cigarette)	%RSD*	Average (ng/cigarette)	%RSD	Average (ng/cigarette)	%RSD
Naphthalene	39.1	7.8	281.8	4.6	1247.7	3.5
Fluorene	21.7	3.3	121.2	8.7	309.2	9.9
Phenanthrene	25.6	4.4	79.2	9.0	182.3	8.8
Anthracene	7.5	6.0	40.8	9.8	90.0	7.0
Fluoranthene	10.9	2.2	40.4	6.8	96.2	3.5
Pyrene	6.2	1.9	25.7	4.3	54.6	1.5
Benzofluorene	4.0	3.5	27.6	9.7	54.3	2.9
Benzanthracene	2.1	4.3	8.6	4.1	18.7	7.6
Chrysene	3.0	4.1	12.2	5.0	25.0	5.8
Benzofluoranthene	2.2	4.7	7.4	3.4	18.1	4.1
BEP	0.9	5.8	3.6	6.4	8.5	7.5
BAP	1.1	0.7	4.5	2.6	10.5	5.9
Perylene	0.1	9.7	0.5	8.7	1.3	7.7
Dibenzanthracene	0.06	16.4	0.2	18.2	0.5	10.2
Benzoperylene	0.4	9.1	0.9	4.4	2.1	4.6
Total PAH (ng)	**124.9**		**654.6**		**2119.1**	
TPM (mg)	**2.1**		**10.9**		**24.4**	
PAH per TPM (ng/mg)	**58.2**		**59.9**		**86.8**	

* RSD, relative standard deviation.

Numerous techniques have been developed for the quantitative analysis of BAP in cigarette smoke. The measurement of BAP concentration in the particulate-phase smoke of 1R4F Kentucky reference cigarettes has been frequently reported. The reported BAP levels from 1R4F cigarette smoke are listed in Table IV.

There are discrepancies in reported BAP concentrations from 1R4F cigarette smoke. It should be noted that different analytical techniques might cause differences in reported BAP concentrations. However, the data should be within a lim-

ited range of variations. The reported data in Table IV shows a wide range of variations. Some of the possible causes for the discrepancies are listed below.

All reported data were measured for mainstream smoke. There were differences in the technique of smoke collection. As a standard, the particulate-phase smoke was collected on a smoke pad under FTC conditions. Some of the data were obtained by measuring mainstream smoke using an impinger smoke trap. The data from mainstream whole smoke could be higher than the data from particulate-phase smoke.

Some data were obtained by HPLC techniques. Positive validation for the separation of BAP from BEP has to be established. The interference of BEP may increase the reported BAP concentration.

Generally, the LR-SIM mode is used when a GC–MS technique is adopted for PAH detection. The interference of a fragment ion at *m/z* 252 from long-chain hydrocarbons with the molecular ion of BAP at *m/z* 252 must be positively excluded.

Quantitative analysis of BAP is typically based on the calculation of an RF. Both BAP and deuterated BAP-d_{12} should have exactly the same recoveries, in theory, to ensure the accuracy of results based on the RF. When the recovery for all PAHs is uniform and high enough, a slight recovery difference between BAP and BAP-d_{12} would not affect BAP quantitation. If the recovery for PAHs is different and very low, a slight recovery difference between BAP and BAP-d_{12} would significantly alter the BAP results.

The newly developed SPE technique for PAH analysis avoids all the possible inferences mentioned. It can be assumed that the data reported using this technique are accurate. The BAP concentration in the particulate-phase smoke of 1R4F Kentucky reference cigarettes under FTC smoking conditions should be in the range of 4.5 to 5.0 ng/cigarette, considering technique variations.

The extraction and cleanup of PAHs from particulate-phase smoke using an SDE technique has been previously reported and extensively evaluated (3). The comparison with the levels of other PAHs determined by the SPE method was in good agreement with the levels of PAHs measured by the SDE method. This is shown in Table V for the 1R4F Kentucky reference cigarette.

A recovery study has been conducted for 22 PAHs (15 analytes and 7 labeled ISs). For this purpose, 100 ng of each PAH standard (equivalent to 5 ng/cigarette) was spiked on a pad that was extracted as described in the Experimental section. Two different SPE columns, Bond Elut C18 and Bond Elut CH (both Varian products), were evaluated for PAH extraction. The C18 column had a recovery range of 46.5% to 67.5% for the 22 PAH standards. The average recovery was 59.8%, which was considered very good for a SPE procedure. The CH column showed even better results. The range of recovery was from 90.7% to 106.4% and the average recovery was 97.1%. The recoveries for different PAHs are shown in Figure 6.

Table IV. Reported BAP Levels From 1R4F Cigarette Smoke

Concentration (ng/cigarette)	Year	Reference no.
6.6	1985	4
6.4	1988	5
9.2	1991	6
8.5	1993	7
5.3–8.2	1993	8
7.9	1997	3
5.0	1997	9
7.6	2000	10
4.6	2000	11
4.6	2001	this study

Table V. Levels of Various PAHs Measured in the Mainstream Smoke of a 1R4F Kentucky Reference Cigarette by the SDE Method* and the Method in This Study

PAH	SPE (ng/cigarette)	SDE (ng/cigarette)
Naphthalene	281.8	290.7
Fluorene	121.2	108.1
Phenanthrene	79.2	92.1
Anthracene	40.8	48.3
Fluoranthene	40.4	55.1
Pyrene	25.7	31.1
Benzofluorene	27.6	23.3
Benzanthracene	8.6	8.4
Chrysene	12.2	12.5
Benzo[b,k]fluoranthene	7.4	11.8
BEP	3.6	6.5
BAP	4.6	6.8
Perylene	0.5	0.9
Dibenzanthracene	0.2	BDL†
Benzoperylene	0.9	BDL

* Reference 10.
† BDL, below detection limit.

Conclusion

An SPE procedure is the technique of choice for the quantitative analysis of PAHs in particulate-phase smoke. The advantages for this technique are high recovery rate, good repeatability, and low detection limit. The operation of this technique is simple,

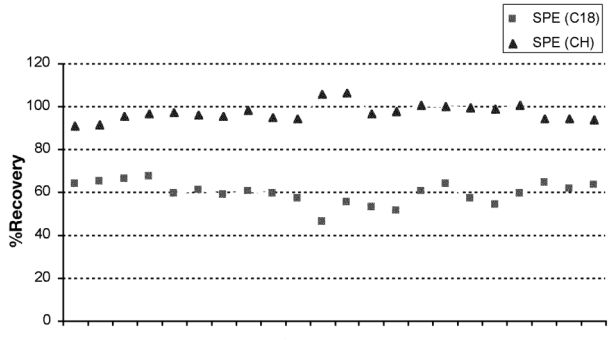

Figure 6. Comparison of recoveries between two different SPE phases.

easy, and fast. No sample heating and evaporating is involved in this technique to avoid the loss of analytes. The only drawback for SPE is that it can be a labor-intensive procedure. However, the use of an automated sample preparation system could eliminate this problem.

The use of GC–HR-MS detection proved to be a good sample cleanup process using SPE. The calibration showed a linear dynamic range from 0.125 to 50 ng/cigarette. The detection limit for a BAP concentration was at 0.1 ng/cigarette. The BAP concentration in the particulate-phase smoke of 1R4F Kentucky cigarettes (determined to be 4.6 ng/cigarette) was in agreement with some of the more recently reported literature data (9,11).

Acknowledgments

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References

1. R.R. Baker. The kinetics of tobacco pyrolysis. *Thermochim. Acta* **17:** 29–63 (1976).
2. D. Hoffman and S.S. Hecht. "Advance in Tobacco Carcinogenesis". In *Chemical Carcinogenesis and Mutagenesis*. C.S. Cooper and P.L. Grover, Eds. Springer-Verlag, Berlin, Germany, 1990, pp. 63–102.
3. G. Gmeiner, G. Stehlik, and H. Tausch. Determination of seventeen polycyclic aromatic hydrocarbons in tobacco smoke condensate. *J. Chromatogr. A* **767:** 163–69 (1997).
4. B.A. Thomkins, R.A. Jenkins, W.H. Griest, R.R. Reagan, and S.K. Holladay. Liquid chromatographic determination of benzo[a]pyrene in total particulate matter of cigarette. *J. Assoc. Off. Anal. Chem.* **68:** 935–40 (1985).
5. C.H. Risner. The determination of benzo[*a*]pyrene in the total particulate matter of cigarette smoke. *J. Chromatogr. Sci.* **26(3):** 113–20 (1988).
6. C.H. Risner. The determination of benzo[a]pyrene and benzo [a]anthracene in mainstream and sidestream smoke of Kentucky reference cigarette 1R4F and a cigarette which heats but not burn tobacco: a comparison. *Beitr. Tabakforsch.* **15:** 11–17 (1991).
7. J. Dumont, F. Laroque-Lazure, and C. Iorio. An alternative isolation procedure for the subsequent determination of benzo(a)pyrene in total particulate matter of cigarette smoke. *J. Chromatogr. Sci.* **31(9):** 371–74 (1993).
8. W.H. Evans, N.C. Thomas, M.C. Boardman, S.J. Nash, and J. Stephen. Relationship of polycyclic aromatic hydrocarbon yields with particular matter (water and nicotine free) yields in mainstream and sidestream cigarette smoke. *Sci. Total Environ.* **136:** 101–109 (1993).
9. M.F. Borgerding, J.A. Bodnar, H.L. Chung, P.P. Mangan, C.C. Mossison, C.H. Risner, J.C. Rogers, D.F. Simmons, M.S. Uhrig, F.N. Wendelboe, D.E. Wingate, and L.S. Winkler. Chemical and biological studies of a new cigarette that primarily heats tobacco, Part I. Chemical composition of mainstream smoke. *Food Chem. Toxicol.* **36:** 169–82 (1997).
10. J.B. Forehand, G.L. Dooly, and S.C. Moldoveanu. Analysis of polycyclic aromatic hydrocarbons, phenols and aromatic amines in particulate phase cigarette smoke using simultaneous distillation and extraction as a sole sample cleanup step. *J. Chromatogr. A* **898:** 111–24 (2000).
11. T.A. Chepiga, M.J. Morton, P.A. Murphy, J.T. Avalos, B.R. Bombick, D.J. Doolittle, M.F. Borgeding, and J.E. Swauger. A comparison of mainstream and sidestream smoke chemistry and mutagenicity of a representative sample of the US cigarette market with two Kentucky reference cigarettes (K1R4F and K1R5F). *Food Chem. Toxicol.* **38:** 949–62 (2000).
12. H.C. Pillsbury, C.C. Bright, K.J. O'Connor, and F.W. Irish. Tar and nicotine in cigarette smoke. *J. Assoc. Off. Anal. Chem.* **52:** 458–62 (1969).
13. *Routine Analytical Cigarette Smoking Machine—Definitions and Standard Conditions*. International Standard Organization, Geneva, Switzerland, Vol. 3308, 1991, pp. 1–3.
14. *Tobacco and Tobacco Products—Atmosphere for Conditioning and Testing*. International Standard Organization, Geneva, Switzerland, Vol. 3402, 1991, pp. 1–2.
15. *Cigarettes—Determination of Total and Nicotine Free Dry Particulate Matter Using a Routine Analytical Smoking Machine*. International Standard Organization, Geneva, Switzerland, Vol. 4387, 1991, pp. 1–14.

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