

Quantitative Determination of Naphthalenes in Tobacco Smoke by Gas Chromatography*

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INTRODUCTION

In previous papers from this laboratory, analytical methods were presented for the isolation and identification of the complex mixture of polynuclear aromatic hydrocarbons (PAH) in cigarette smoke (1–6). During that work, we developed a method for the quantitative analysis of three-ring and larger PAH in small quantities of cigarette smoke condensate (CSC) (Figure 1) (2, 5). The final gel adsorption chromatographic step not only isolated the PAH but also separated the multialkylated PAH from the monomethyl and parent PAH (GF-B and GF-C, respectively, Figure 2) (7).

Dividing the complex smoke PAH fraction into two cuts allowed the quantitation of the major parent PAH isolated in GF-C (Figure 3). Recovery studies with ^{14}C -benzo[a]pyrene (BaP) and ^{14}C -anthracene indicated quantitative recovery of these smoke PAH (5). However, our work and that of others indicated that the levels of naphthalenes and fluorenes in the chromatograms (Figure

3) were less than the actual levels present in CSC (2, 8, 9, 10). The high volatility of the naphthalenes suggested their loss during solvent reduction (9). It became apparent that special solvent removal techniques must be developed for quantitative isolation of these compounds. We now describe our modification of the four-step PAH isolation scheme such that all the major PAH from naphthalene to coronene, including their methyl derivatives, can be quantitatively isolated.

EXPERIMENTAL

Cigarette Smoke Condensate Collection

University of Kentucky reference cigarettes (type 1R1, 85 mm non-filter) were conditioned at 60% relative humidity for 48 hours and were smoked to a 30 mm butt length on a 30-port Borgwaldt⁺ smoking machine under the standard smoking conditions of 1 puff/min, 2-second puff duration, and a puff volume of 35 ml (11, 12). The condensate was collected in two dry-ice traps with a glass wool plug in the last trap.

Chromatography

Silicic acid (SA) and gel chromatography were performed as before (5), but a 30 ml injection loop was used.

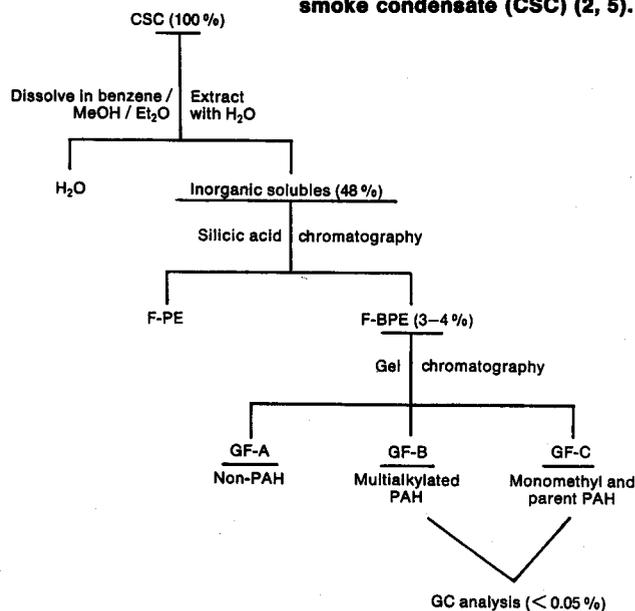
Gas Chromatographic Standards

Except for 2-methylnaphthalene, the PAH standards used for determination of gas chromatographic (GC) response data were purified by chromatography on a short SA column with benzene/petroleum ether (B/PE, 1/3, v/v) followed by recrystallization. The 2-methylnaphthalene was used as obtained from Eastman Kodak Co.

Gas Chromatography

GC analyses were performed with a Hewlett-Packard model 5840A gas chromatograph equipped with a 15 ft. × 1/8 in. stainless steel column, packed with 5% Dexsil 300 GC on 100/120 mesh Chromosorb W-AW. For Figure 3, the temperature program was 90 °C for 10 min, 90–325 °C at 2°/min, and held at 325 °C for 45 min. For the chromatogram shown in Figure 4, the temperature program was 70–250 °C at 2°/min. Helium flow was 40 ml/min at 90 °C; injector temperature, 290 °C; flame ionization detector temperature, 350 °C.

Figure 1. Four-step polynuclear aromatic hydrocarbon (PAH) isolation scheme for small quantities of cigarette smoke condensate (CSC) (2, 5).



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+ Reference to a company or product name does not imply approval or recommendation by the United States Department of Agriculture.

Isolation Procedure

Cigarettes (270) were machine smoked and materials adhering to the glass connecting tubes in the trapping system were dissolved alternately with methanol and benzene into the traps. After warming to room temperature, the CSC solutions in the traps were quantitatively transferred into a separatory funnel with alternating portions of benzene, methanol, and ethyl ether (total volumes 200, 100, 100 ml, respectively). The organic solution was washed with glass-distilled water (3×200 ml), and the aqueous portion was cross-extracted with 1:1 benzene/ethyl ether (3×50 ml). The organic layers were combined and transferred to a 2-liter round-bottom flask. Isooctane (IO, 200 ml) was added, and the flask was placed on a modified commercial distillation apparatus (MCDA) developed by Schepartz et al. (13) at our laboratory. The volume was reduced until most of the benzene had been removed (final volume about 2.0 ml). After the addition of 40 g of silicic acid (SA), the remaining solvent was removed on a rotary evaporator (40°C) until a semi-solid mixture of IO, SA, and sample remained. This residue was slurried with 150 ml of petroleum ether (PE) and added to the top of a 100 g SA column. The column was eluted with 400 ml PE to yield fraction F-PE. (This fraction should be colorless. The elution of colored material means that the SA was not sufficiently active to retain the low molecular weight PAH.) The round-bottom flask was rinsed with 100 ml of B/PE (1:3, v/v), and the rinsings were added to the column. The column was eluted with 900 ml of the B/PE solvent mixture to yield fraction F-BPE, which was collected directly into a 2000 ml round-bottom flask. Several boiling stones were added (high purity, amphoteric Alundum granules), and the volume was reduced to about 25 ml with MCDA. The residue was quantitatively transferred to a 50 ml pear-shaped flask. A small magnetic stirring bar was added for agitation and the flask was connected to a Kontes' Bantum-ware vacuum-jacketed Vigreux distillation head. The flask was heated with a heating mantle and the temperature of the distillation was monitored with an immersion thermometer at the top of the Vigreux column. Sample volume was reduced to about 3 ml and the residue was quantitatively transferred with benzene to a 5 ml volumetric flask. A 3 ml sample of

the F-BPE solution was injected onto the gel system, and sixty 8 ml fractions were collected. Gel fractions 35–60 (Figure 2) were combined into a 2000 ml round-bottom flask to yield the PAH isolate. The volume was reduced to about 25 ml with the MCDA, as above, and the residue (PAH isolate) was quantitatively transferred to a 50 ml pear-shaped flask. Pentatriacontane (C_{35}) was added as internal standard, and the volume was reduced by distillation with a Vigreux column to 3 ml as above. At this point, concentration of the C_{35} internal standard should be about $0.5 \mu\text{g}/\mu\text{l}$. Three to eight microliters of the PAH solution were used for GC analysis.

RESULTS AND DISCUSSION

Major losses of volatile, small-ring PAH occurred in our previous method during conventional solvent reduction (rotary evaporation and nitrogen blow-down) to volumes suitable for gel and gas chromatography. Even with solvent removal by efficient distillation columns, considerable losses of ^{14}C -naphthalene occurred, when solutions were reduced below 2.5 ml (Table 1). Therefore, this was

Table 1. Recovery of ^{14}C -naphthalene after solvent reduction.

Volume reduction*	Recovery of ^{14}C -naphthalene (%)
1000 ml to 2.5 ml	99.2
1000 ml to 1.0 ml	87.3
250 ml to 2.0 ml	96.0
250 ml to 0.5 ml	65.0

* Volumes were reduced with the modified commercial distillation apparatus (MCDA) and a vacuum-jacketed Vigreux distillation head.

set as the lowest level for solvent reduction. At a final volume of 2.5 ml, the small-ring PAH could still be readily analyzed in CSC obtained from 200 cigarettes. The recovery of naphthalene from CSC was verified with ^{14}C -naphthalene at each step in the procedure, including the solvent reduction step (Table 2). Quantitative recovery was achieved in the water extraction, the SA column chromatography, and the gel filtration steps; and it was also quantitative for the solvent-reduction steps. Total naphthalene recovery of 91% (Table 2) for the entire fractionation scheme was acceptable. When counting at each step in the process, lower recoveries could result from

Table 2. Recovery of ^{14}C -naphthalene (average of two analyses).

Steps in PAH isolation	Recovery/step (%)	Recovery (%)
Cigarette smoke condensate	100	100
Organic solubles	99	99
Silicic acid	99	98
Solvent reduction (to 2.5 ml)	96	95
Gel filtration	100	95
Solvent reduction (to 2.5 ml)	95	91

Figure 2. Absorbance curves for fraction F-BPE and for PAH standards used to define limits for gel chromatography fraction cuts (8). (GF-A contained non-PAH material).

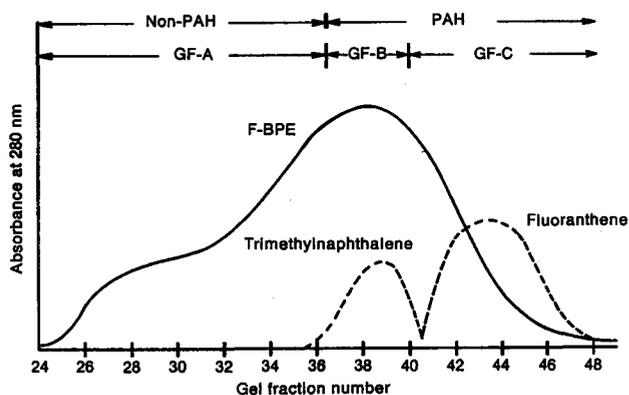


Figure 3. Gas chromatograms from a previous analysis of PAH from reference cigarettes (2, 5).

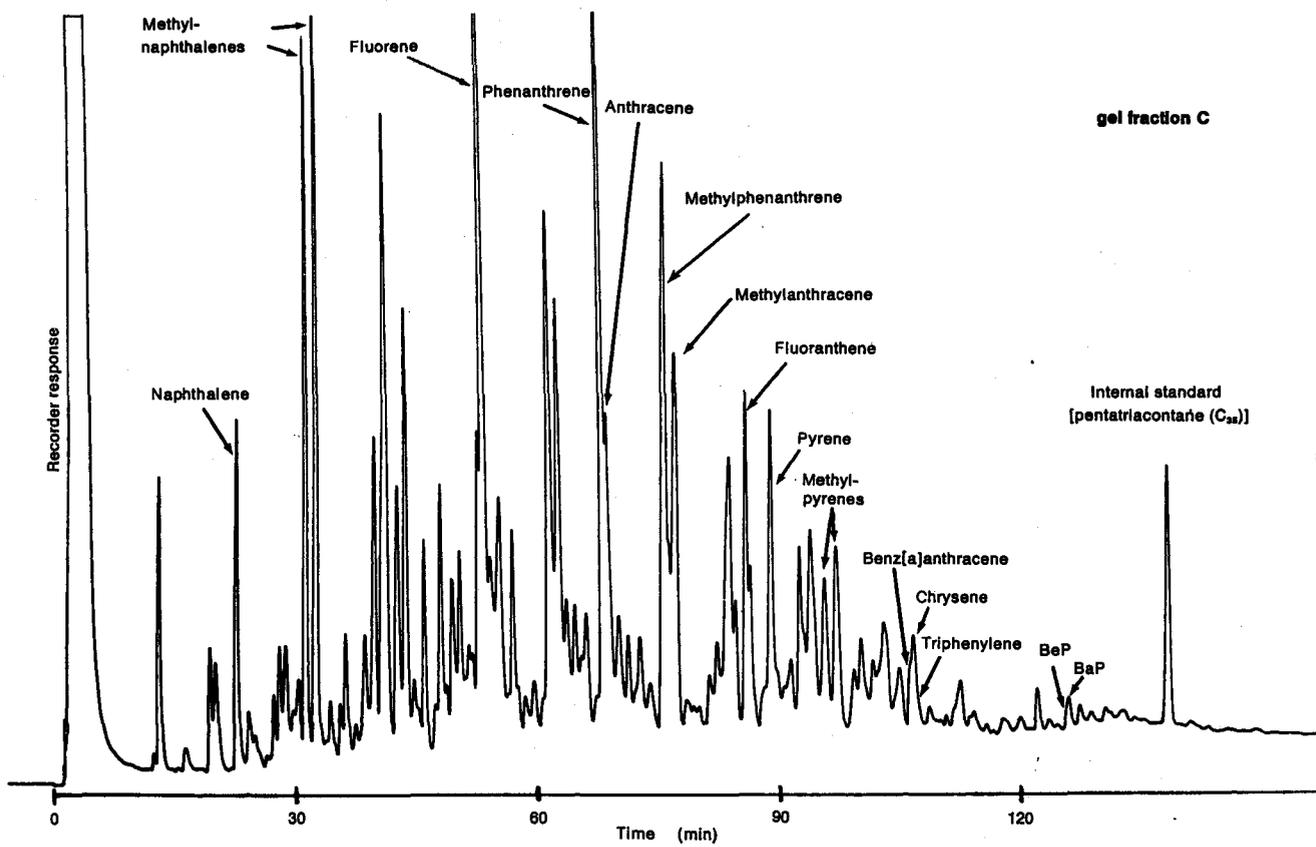
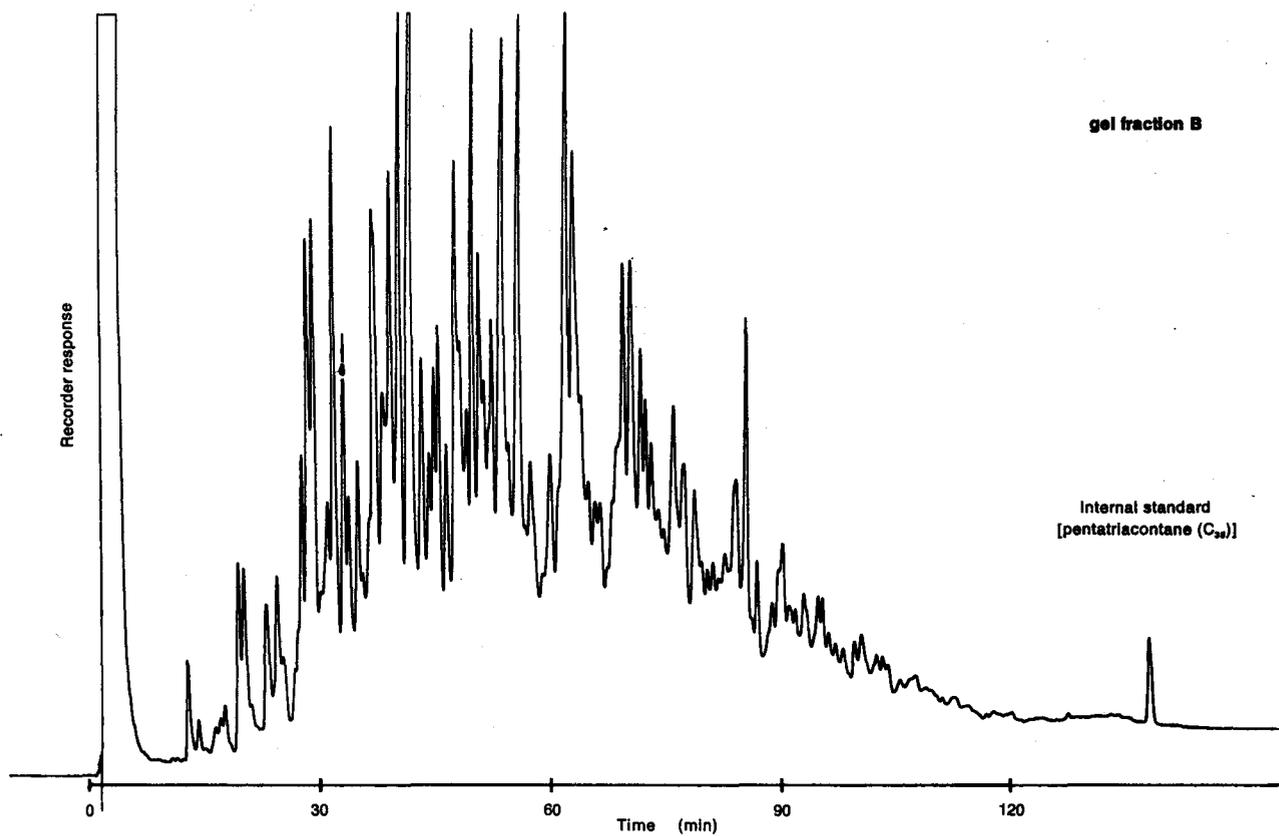


Table 3. Major components identified in "volatile PAH" isolate.

1. Indene
2. Dimethylstyrenes, methylbenzo[b]furans
- 3, 4. Methylindenes
5. Naphthalene
6. Dimethylbenzo[b]furans
7. Dimethylindenes, ethylindenes, ethyldimethylindenes
8. 2-Methylnaphthalene
9. 1-Methylnaphthalene
- 8-13. Trimethylindenes, trimethylbenzo[b]furans
12. Tetramethylbenzo[b]furans
13. Biphenyl, tetramethylbenzo[b]furans, tetramethylindenes
14. Tetramethylbenzo[b]furans, tetramethylindenes
15. 1- & 2-Ethynaphthalene, tetramethylbenzo[b]furans, tetramethylindenes
16. 2,6- & 2,7-Dimethylnaphthalene, 1-vinylnaphthalene
17. 1,3-, 1,7- & 1,6-Dimethylnaphthalene, 2-vinylnaphthalene
18. 2,3-, 1,4- & 1,5-Dimethylnaphthalene, isopropylnaphthalene
19. Acenaphthylene, 1,2-dimethylnaphthalene
20. 3-Methylbiphenyl
21. 4-Methylbiphenyl
22. Acenaphthene, 1,8-dimethylnaphthalene, bibenzyl
23. Ethylmethylnaphthalenes, trimethylnaphthalenes
24. Dibenzofurans, trimethylnaphthalenes
- 25, 26. Trimethylnaphthalenes
27. Tri & tetramethylnaphthalenes
28. Trimethylnaphthalenes
29. Trimethylnaphthalenes, methylacenaphthylene

Table 3 (contd.)

30. Methylacenaphthylenes
31. Fluorene
32. Methylacenaphthylenes, 9-methylfluorene
33. 1-Methylidibenzofuran, tetramethylnaphthalenes
34. 2- & 4-Methylidibenzofuran, tetramethylnaphthalenes
- 35, 36. Dimethylacenaphthalenes, tetra & pentamethylnaphthalenes
37. Trimethylacenaphthalenes, pentamethylnaphthalenes
38. 2- & 3-Methylfluorene, dimethylacenaphthylenes, pentamethylnaphthalenes
39. 1- & 4-Methylfluorene, dimethylacenaphthylenes
- 40-42. Trimethylacenaphthylenes, dimethylacenaphthylenes, dimethylbenzofurans

losses during the many transfers of material. Thus, when determined only at the point of GC analysis, recoveries were about 95%.

The use of radio-labeled compounds, such as ¹⁴C-naphthalene and ¹⁴C-phenanthrene, as internal standards in isolation schemes for the naphthalenes has been advocated (9). However, the use of our modified four-step isolation procedure would assure quantitative PAH recovery and reproducibility and would avoid the inconvenient use of radio-labeled tracers.

The GC profile of the volatile, small-ring PAH from Kentucky 1R1 cigarettes isolated by our modified method is shown in Figure 4. The major volatile components are listed in Table 3. The chromatogram represents about half of the total PAH profile given in Figure 3. Note the high levels of alkylated benzenes (toluene, xylenes, etc.) and styrenes eluting before indene (Peak 1 in Figure 4). In contrast, the majority of these volatile PAH are absent in the GC profiles (Figure 3) of gel fractions obtained by our previous isolation method. A complete identification of the components of GF-B and GF-C has been reported (1, 4, 6).

Figure 4. Gas chromatogram of "volatile PAH" isolate derived from smoke condensate of reference cigarettes. (GC peaks are identified in Table 3.)

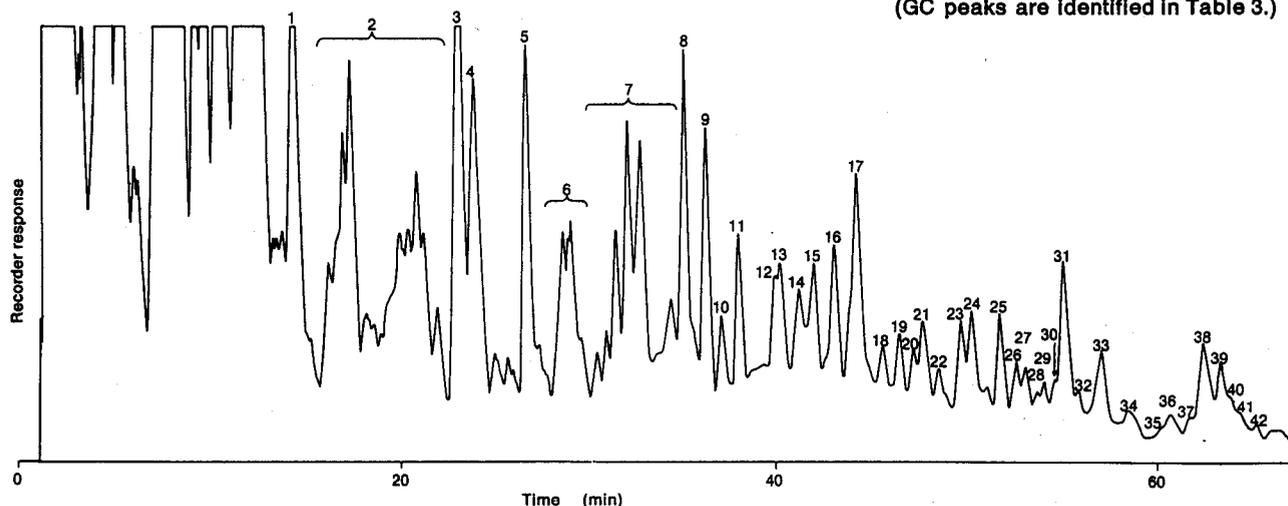


Table 4. Selected PAH levels in cigarette smoke condensate from 1R1 cigarettes smoked to a 30 mm butt length.

Polycyclic aromatic hydrocarbons	amount (ng/cigarette)
Naphthalene	2560.0
2-Methylnaphthalene	2490.0
1-Methylnaphthalene	1930.0
Fluorene	1640.0
Phenanthrene + anthracene	440.0 (5)
Methylphenanthrene + methylanthracene	1020.0 (5)
Fluoranthene	148.0 (5)
Pyrene	139.0 (5)
Methylpyrene	199.0 (5)
Benz[a]anthracene + chrysene + triphenylene	86.0 (5)
Benzo[a]pyrene + benzo[e]pyrene	25.0 (5)
Perylene	3.0 (3)
Indenopyrene	6.0 (3)
Benzo[ghi]perylene	5.0 (3)
Anthanthrene	3.0 (3)
Methylbenzo[ghi]perylene	5.0 (3)
Coronene	1.0 (3)

The levels of selected PAH in smoke condensate from Kentucky 1R1 reference cigarettes are listed in Table 4. These data were obtained based upon peak areas of sample components versus peak area of our internal standard (C_{38}), taking into consideration the relative responses of each component. The quantitative data on naphthalene to fluorene in Table 4 is the average of two complete analyses. The average relative percent standard deviation for these two analyses was 5.1%. Values are given for PAH in ng/cigarette over the total PAH range from naphthalene to coronene. Our value of 2.5 $\mu\text{g}/\text{cigarette}$ for naphthalene for a Kentucky 1R1 cigarette smoked to a 35 mm butt length is in good agreement with 2.76 $\mu\text{g}/\text{cigarette}$ found by *Schmeltz et al.* (9) when smoking to a 23 mm butt length. However, their levels of alkylated PAH were lower. They obtained a value of 2.22 $\mu\text{g}/\text{cigarette}$ for the combined amounts of 1-methyl and 2-methylnaphthalenes, as compared with our value of 4.42 $\mu\text{g}/\text{cigarette}$. We believe that this difference is due to the fact that certain PAH, especially alkylated PAH, have unfavorable partition coefficients in organic solvent-solvent partition systems (14). Our value of 1640 ng/cigarette for fluorene is also much higher than the 782 ng/cigarette obtained by *Hoffmann et al.* (15) using a less rigorous isolation procedure.

The entire range of PAH from naphthalene to coronene can now be quantitatively isolated by this modified four-step procedure. This solvent reduction procedure may also be adapted for the quantitative isolation of other volatile compounds from CSC, while the total methodology should be most applicable to the analysis of environmental samples containing volatile and non-volatile compounds, such as PAH.

SUMMARY

Our previously developed method for the determination of three-ring and larger polynuclear aromatic hydrocarbons (PAH) of cigarette smoke was modified to allow quantitative determination of the more volatile PAH. Recovery tests with ^{14}C -naphthalene showed that this smoke PAH was being lost to a large extent during the usual solvent evaporation steps. Special solvent removal techniques, using very efficient distillation equipment, were developed and tested to quantitatively recover the naphthalenes. The gas chromatographic profile for the volatile PAH, including naphthalenes, fluorenes, and related compounds, is discussed in relation to the modified procedure. Quantitative values for some of the PAH of cigarette smoke are given. The developed solvent removal and concentration techniques should be most applicable to the analysis of environmental samples containing volatile compounds.

ZUSAMMENFASSUNG

Das von den Autoren früher entwickelte Verfahren zur Bestimmung der polycyclischen aromatischen Kohlenwasserstoffe (PAH) mit drei und mehr Ringen im Zigarettenrauch wurde für den quantitativen Nachweis der flüchtigeren polycyclischen aromatischen Kohlenwasserstoffe modifiziert. Bei Rückgewinnungsversuchen mit ^{14}C -Naphthalin hatte sich gezeigt, daß dieser Rauchinhaltsstoff während der üblichen Lösungsmittelverdampfung weitgehend verlorenggeht. Für die Abtrennung der Lösungsmittel wurden unter Benutzung sehr leistungsstarker Destillationsvorrichtungen besondere Techniken entwickelt und an der quantitativen Wiederauffindung der Naphthaline erprobt. Das gaschromatographische Profil der flüchtigen polycyclischen aromatischen Kohlenwasserstoffe, einschließlich der Naphthaline, Fluorene und verwandter Verbindungen, wird im Zusammenhang mit dem modifizierten Verfahren diskutiert. Für einige der polycyclischen aromatischen Kohlenwasserstoffe des Zigarettenrauches werden quantitative Ergebnisse mitgeteilt. Die beschriebenen Abtrennungs- und Konzentrationsverfahren könnten sich bei der Bestimmung der Luftverunreinigung sehr gut zur Untersuchung von Proben der Umgebungsluft auf flüchtige Verbindungen eignen.

RÉSUMÉ

La méthode présentée précédemment pour la détermination des hydrocarbures aromatiques polycycliques (PAH) à trois cycles et plus dans la fumée de cigarette a été modifiée pour la détermination quantitative des PAH plus volatils. Des essais de récupération au moyen de ^{14}C -naphthaline avaient démontré que ce constituant de la fumée disparaît dans une large mesure pendant l'évaporation des solvants. Une technique de distillation très efficace a été mise au point pour l'élimination des solvants, et éprouvée par la récupération quantitative de la naphthaline. Le chromatogramme en phase gazeuse des PAH vola-

tils, comprenant des naphthalines, fluorènes et composés apparentés, est discuté par rapport à la méthode modifiée. Des résultats quantitatifs sont communiqués pour certains PAH de la fumée de cigarette. Les techniques décrites pour l'élimination et la concentration des solvants s'appliquent aisément à l'analyse d'échantillons d'air ambiant contenant des composants volatils.

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Erratum

Page 100, Figure 1:

The fourth line of Figure 1 on page 100 should read "organic solubles (48%)".