Chromatographic Profiling as a Tool in the Comparison and Evaluation of Complex Mixtures

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Abstract

Chromatographic profiling, an application in which all peaks in a chromatogram are examined, is useful as a tool for providing comparative information regarding sample composition and chemical complexity. The utility of profiling is demonstrated in two studies that involve complex cigarette smoke matrices. In the first study, profiling reveals that both the vapor phase and particulate phase fractions of mainstream smoke from a cigarette that primarily heats tobacco are reduced in complexity in comparison with control products. Relative to a 1R4F control product, total chromatographic responses (TCRs) for the tobacco-heating cigarette are reduced by 89% in the vapor phase and 70% (93% if major components are excluded) in the particulate phase. The second study focuses on a cigarette that combines two design technologies: an experimental tobacco blend and a new carbon filter. This study shows that the new filter design is primarily responsible for reducing the complexity of the vapor phase fraction of mainstream smoke while having relatively little effect on the particulate phase fraction. Compared with three commercial products, the mainstream smoke vapor phase TCR is reduced by approximately 60%.

Introduction

Since the introduction of capillary columns, gas chromatography (GC) has been applied as a tool to separate the many components forming the complex cigarette smoke matrix (1–4). Over 4000 tobacco smoke components have been described in the literature (5–7). Typically these methods have focused on the task of identifying specific components of the smoke matrix. More recently, work conducted in our laboratories has focused on using GC information as a basis for more general product comparisons (8).

The goal of chromatographic profiling is to provide, in a single experiment, as much information as possible about the comparative chemical complexity of the samples under study. Whereas many analyses focus on only a handful of analytes, profiling includes information from all peaks in a chromatogram. Chromatographic profiling provides both visual and semiquantitative bases for comparing samples. Total ion chromatograms (TIC) afford quick visual inspections of sample complexity. Semiquantitative measures of sample complexity,

Table I. Description of Cigarettes

Cigarette	Description	Total particulate matter (mg/cigarette)*	
		Average	Standard deviation
1R4F	Research "light"	11.4	0.3
COM-ULT	Commercial "ultra low tar"	6.5	0.1
TOB-HT	Primarily heats, rather than burns, tobacco	4.5	0.3
STD-C	Standard U.S. blend and new carbon filter	13.0	0.1
EXP-C	Experimental tobacco blend and new carbon filter	11.5	0.3
EXP	Experimental tobacco blend and standard cellulose acetate filter	12.5	0.5
FFLT-1	Leading commercial "light"	12.4	0.3
FFLT-2	Leading commercial "light"	12.8	0.3
FFLT-CH	Commercial "light" with a charcoal filter	12.9	0.4

* Averages and standard deviations based on six replicate determinations.

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such as the number of chromatographic peaks above a certain threshold, the combined response of these peaks (PCR), and the total chromatographic response (TRC), all provide means for assessing comparative sample complexities.

Experimental

Cigarettes

Cigarettes used in this study included those purchased from the market (COM-ULT, FFLT-1, FFLT-2, and FFLT-CH), those designed and manufactured in our laboratories (TOB-HT, STD-C, EXP-C, and EXP), and reference product purchased from the Tobacco and Health Research Institute at the University of Kentucky (IR4F) (9). Cigarette descriptions are listed in Table I.

Mainstream smoke collection

Mainstream smoke (the smoke that passes through the mouth end of the cigarette) from 40 cigarettes was electrostatically precipitated using a Heinrich Borgwaldt model RM20/CS 20-port rotary smoking machine (Heinrich Borgwaldt, Hamburg, Germany) equipped with an electrostatic precipitation (EP) smoke trap and operated according to the FTC puffing regimen (one 35-cc puff per minute, 2 s duration) (10). The portion of smoke that passed through the EP trap was collected by bubbling the smoke through a series of impinger traps containing 5 mL of methanol (B&J brand high-purity solvent; American Burdick & Jackson, Muskegon, MI) and 6 g of 3-mm glass beads. The first trap was immersed in an ice water bath, and the subsequent three traps were immersed in a dry ice–isopropanol bath. That fraction of smoke collected in the EP trap is known as the particulate phase, and the fraction of smoke collected in the impinger traps is known as the vapor phase. Total particulate matter (TPM) was determined as the mass of material collected in the EP trap. Blank samples were collected in the same manner as the mainstream smoke samples, except that air instead of smoke was drawn through the trapping system.

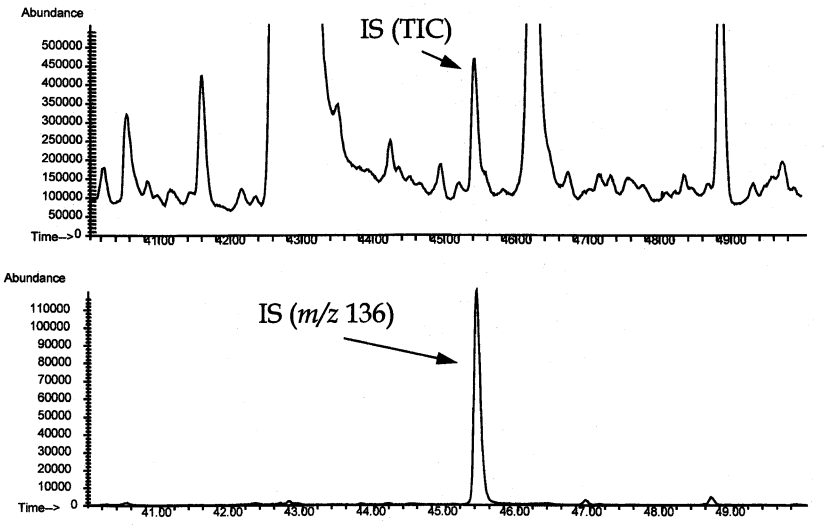

Figure 1. Comparison of the IS in a total ion chromatogram and in an extracted ion (*m/z* 136) chromatogram.

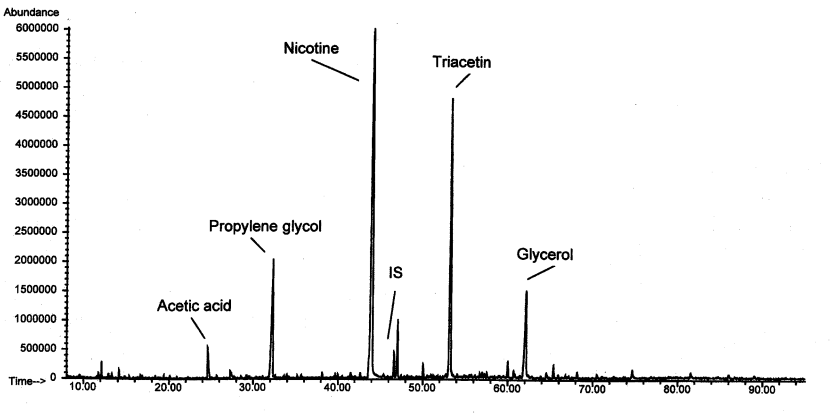

Figure 2. Particulate phase chromatogram showing the IS and the five compounds that typically dominate the profile.

Internal standard preparation

Internal standard (IS) solutions were prepared by adding 181.30 mg (approximately 50 µL) $^2H_7$-quinoline (98 atom %; Cambridge Isotope Laboratories, Woburn, MA) to methanol in a 25-mL volumetric flask and diluting to the mark with methanol.

Sample preparation

Vapor phase samples were prepared by combining the contents of the impinger tubes, adding 100 µL of IS, mixing the solution, and transferring it to autosampler vials, which were then capped. Particulate phase samples were prepared by extracting the contents of the EP trap with 20 mL methanol, adding 100 µL of IS, mixing the solution, and transferring it to autosampler vials, which were then capped. Blank samples were treated in the same manner as smoke samples.

GC–mass spectrometry

Vapor phase smoke components were separated using an HP model 5890 (Hewlett-Packard, Palo Alto, CA) GC equipped with a model 7673A autosampler (Hewlett-Packard) and a DB-1 fused-silica capillary column (60 m × 0.32-mm-i.d., 5-µm film thickness) (J&W Scientific, Folsom, CA). The oven was held isothermal at 35°C for 10 min and then increased at 2.5°C/min to 275°C. The injection port and transfer line temperatures were each 250°C. Helium served as the carrier gas and was maintained at a column head pressure of 14 psi with a split flow of 75 mL/min.

Particulate phase smoke components were

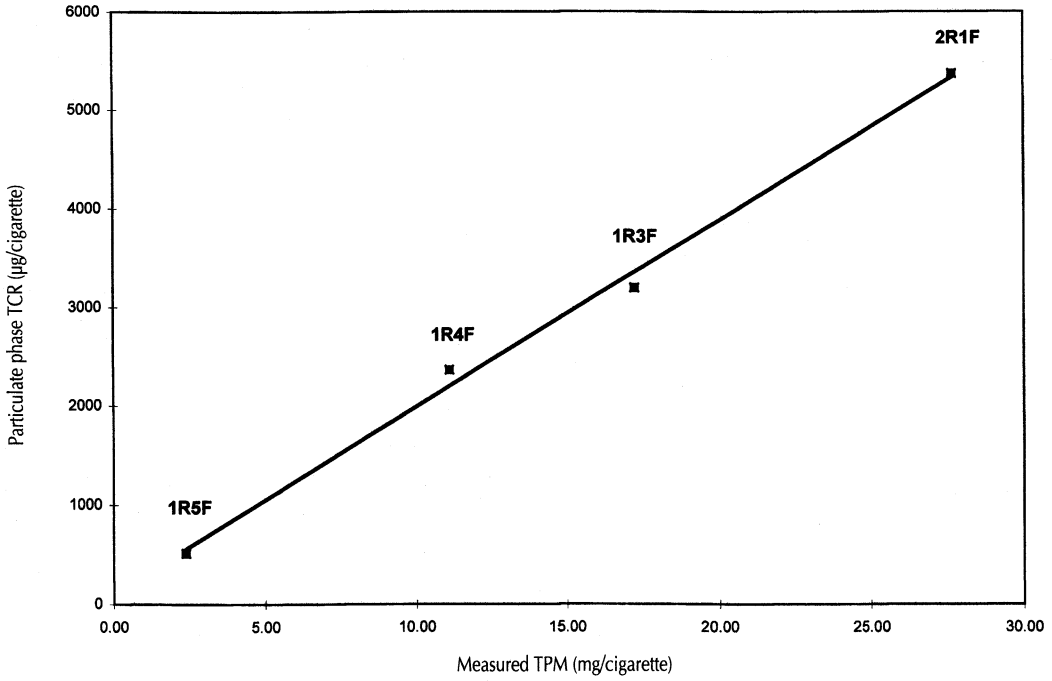

Figure 3. Linearity of particulate phase TCR as a function of measured total particulate matter for a series of reference cigarettes.

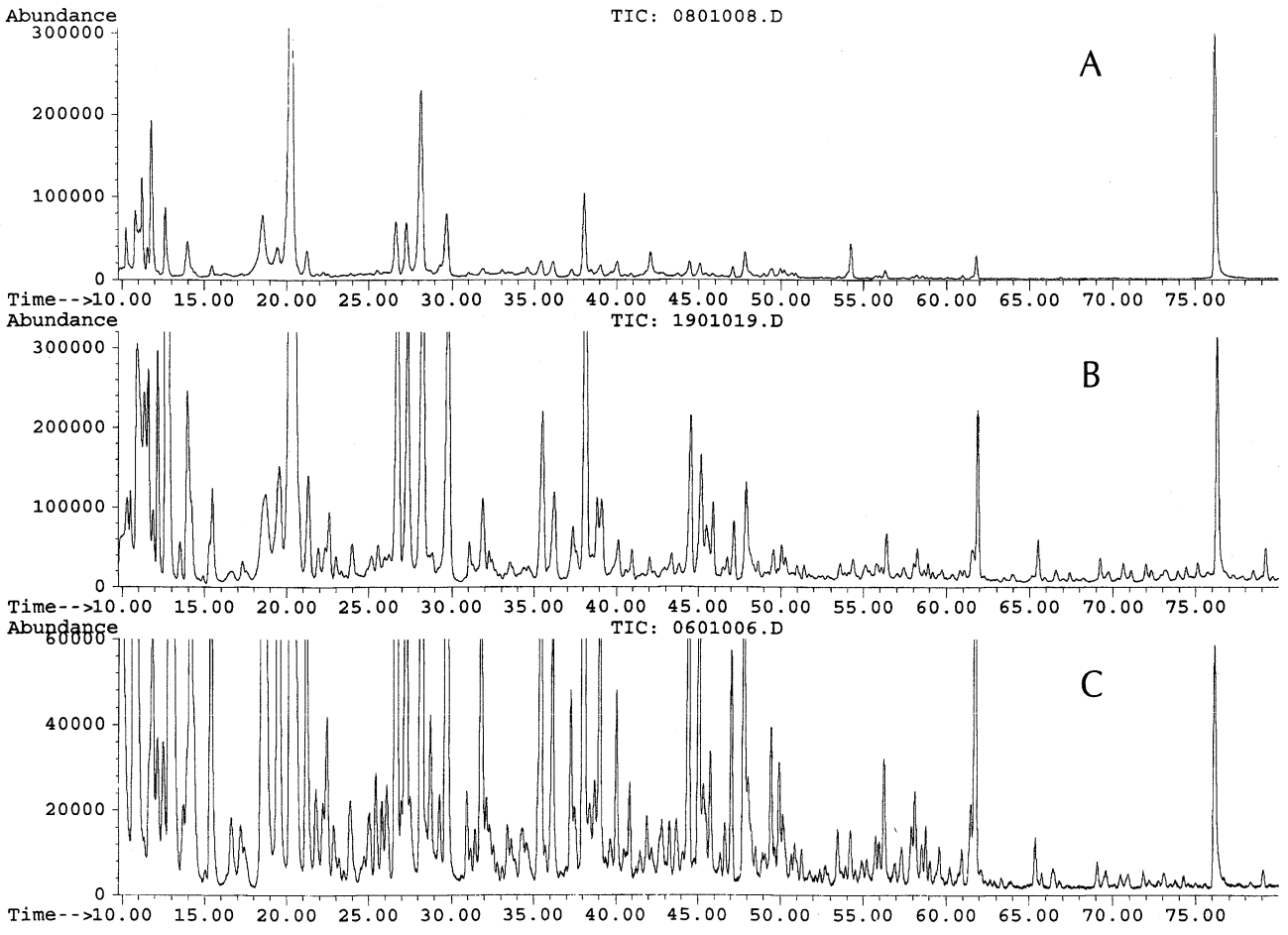

Figure 4. Vapor phase profiles of TOB-HT (A), COM-ULT (B), and 1R4F (C).

separated using a similar GC and a DB-WAX fused-silica capillary column (30 m × 0.25-mm i.d., 0.25-µm film thickness) (J&W Scientific). The oven temperature was held isothermal at 37°C for 2 min and then increased at 2.5°C/min to 230°C. The injection port and transfer line temperatures were both 250°C. Helium served as the carrier gas and was maintained at a column head pressure of 5 psi with a split flow of 75 mL/min.

For both vapor phase and particulate phase samples, autosamplers were used to introduce 1 µL of sample (splitless with split valve opened at 0.5 min) to the respective chromatographic system. Each GC was interfaced to HP model 5972 (Hewlett-Packard) mass spectrometers, which were operated under the manufacturer's "maximum sensitivity autotune" conditions and solvent delays of 10 min. For vapor phase samples, the MS was operated in scan mode from *m/z* 33 to *m/z* 250. For particulate phase samples, the MS was operated in the scan mode from *m/z* 33 to *m/z* 450.

Results and Discussion

The utility of chromatographic profiling was demonstrated in two studies involving new cigarette designs. The first was a cigarette that primarily heated, rather than burned, tobacco

Table II. Summary of Semiquantitative Vapor Phase Data for TOB-HT Study*

	Number of peaks		PCR (µg/cigarette)†		TCR (µg/cigarette)‡	
Cigarette	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
1R4F	113	7	2312	320	2514	325
COM-ULT	79	9	1091	130	1314	139
TOB-HT	29	2	179	57	276	64

* All averages and standard deviations based on six replicate determinations.
† Peak chromatographic response.
‡ Total chromatographic response.

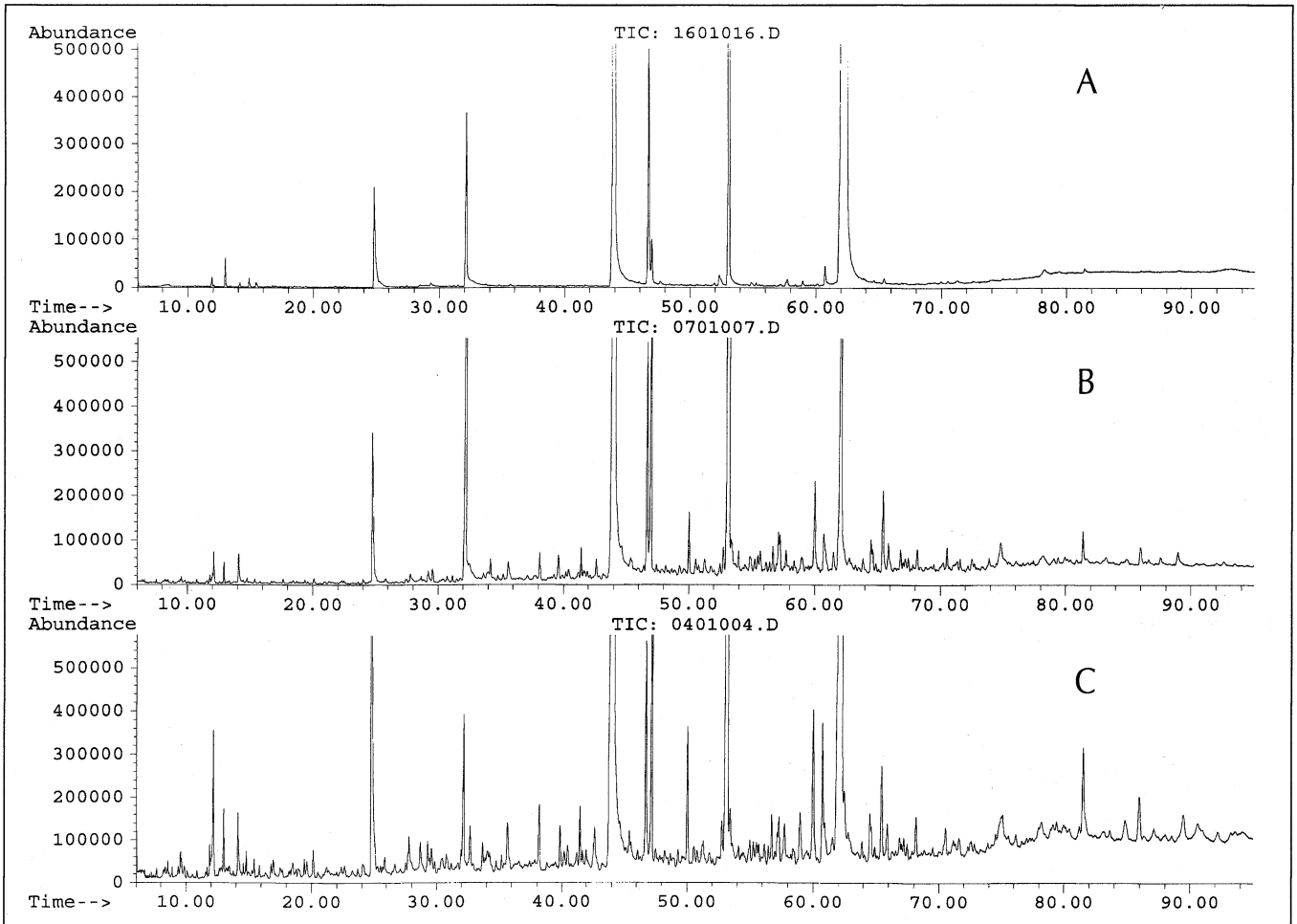

Figure 5. Particulate phase profiles of TOB-HT (A), COM-ULT (B), and 1R4F (C).

(TOB-HT), and the second was a cigarette that contained an experimental tobacco blend and a new carbon filter (EXP-C).

Sample comparisons

Initial assessments of the relative chemical complexities of samples were based on visual comparisons of their chromatographic profiles. These comparisons were complemented with semiquantitative measures that were established to answer the question, "How similar are the chromatograms that are being compared?" Semiquantitative measures included the number of chromatographic peaks, the PCR, and the TCR. These measures were based on the peak area and concentration of the IS and assumed equivalent response factors for all chromatographic peaks and the IS. Limitations imposed by this assumption are described under "Scope and limitations."

The number of chromatographic peaks in each sample was determined by establishing a minimum threshold (0.5 µg/cigarette) for peak detection, adjusting integration parameters to exclude peaks below this threshold, and integrating the chromatograms. The 0.5 µg/cigarette threshold is a practical limitation of the method. Accurate integration of peaks below this threshold is problematic. Any peaks found in the chromatograms

Table III. Summary of Semiquantitative Particulate Phase Data for TOB-HT Study*

	Number of peaks		PCR (µg/cigarette)†		TCR (µg/cigarette)‡		PCR (µg/cigarette)§		TCR (µg/cigarette)§	
Cigarette	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
1R4F	186	6	1551	80	2319	164	500	18	1269	126
COM-ULT	97	9	963	55	1430	69	214	28	681	49
TOB-HT	10	1	614	40	697	39	6	2	89	19

* All averages and standard deviations based on six replicate determinations.
† Peak chromatographic response.
‡ Total chromatographic response.
§ Excluding acetic acid, propylene glycol, nicotine, triacetin, and glycerol.

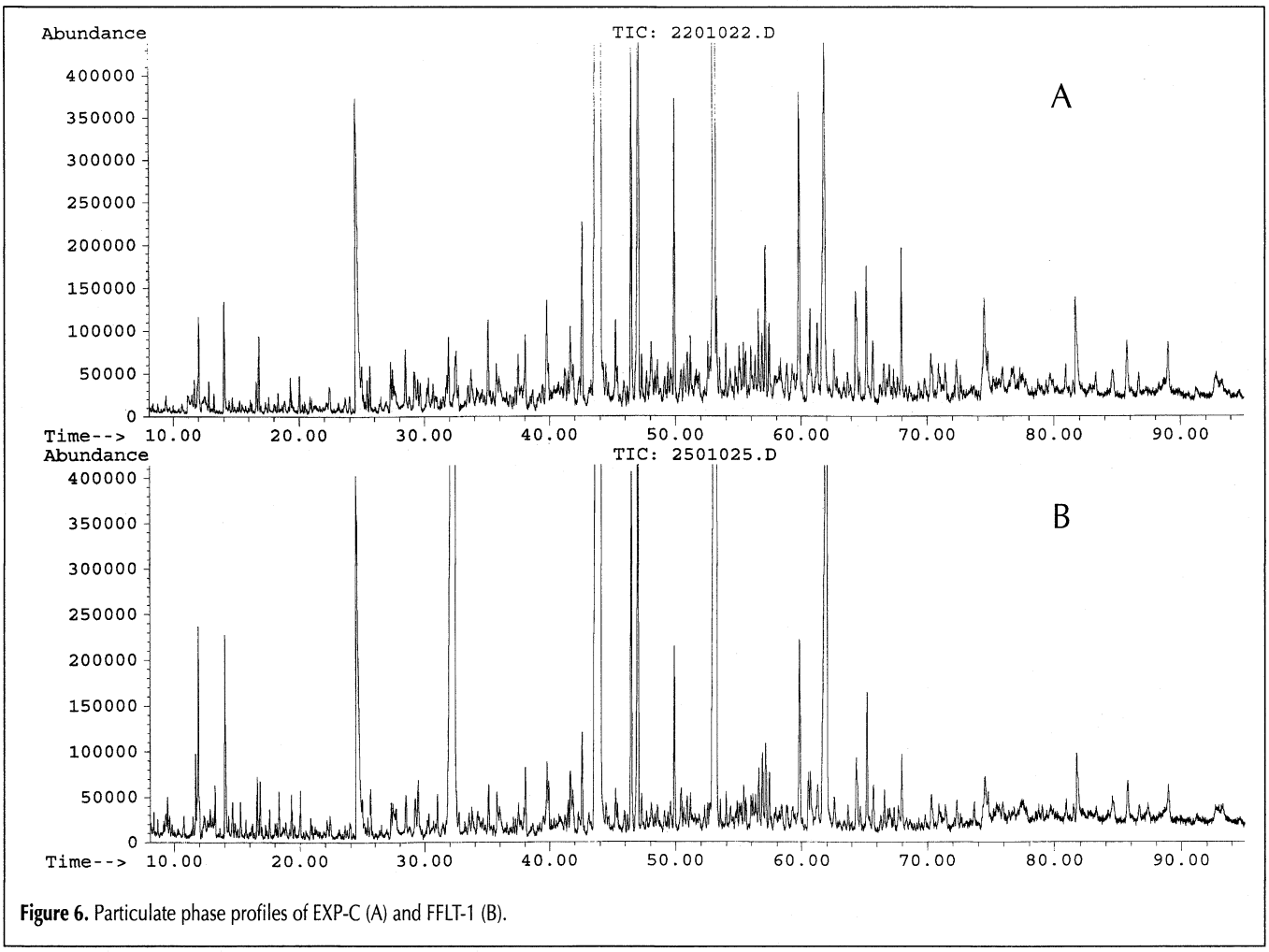

Figure 6. Particulate phase profiles of EXP-C (A) and FFLT-1 (B).

of blank samples were excluded from this calculation.

The PCR for each sample was determined by summing the responses for each peak (as defined by the number of peaks calculation) based on the IS area and concentration.

For each sample, TRC was determined by subtracting a blank sample chromatogram from the chromatogram of interest. The subtracted chromatogram was then manually integrated with the baseline set at zero. CRs were based on IS areas and concentrations.

Determination of IS area by extracted ion

The complexity of chromatographic profiles of cigarette smoke made accurate integration troublesome, primarily due to difficulties in properly assigning peak baselines. The area of

Table IV. Summary of Semiquantitative Particulate Phase Data for EXP-C Study*

	Number of peaks		PCR (µg/cigarette)†		TCR (µg/cigarette)‡		PCR (µg/cigarette)§		TCR (µg/cigarette)§	
Cigarette	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
STD-C	185	6	1625	95	2092	107	667	49	1134	57
EXP-C	183	5	1415	71	1864	74	673	39	1122	42
EXP	205	10	1697	69	2201	73	738	43	1242	48
FFLT-1	176	7	1719	70	2128	97	531	43	940	65
FFLT-2	181	12	1792	38	2257	32	569	50	1034	21
FFLT-CH	198	15	1761	141	2230	180	707	107	1176	149

* All averages and standard deviations based on six replicate determinations.
† Peak chromatographic response.
‡ Total chromatographic response.
§ Excluding acetic acid, propylene glycol, nicotine, triacetin, and glycerol.

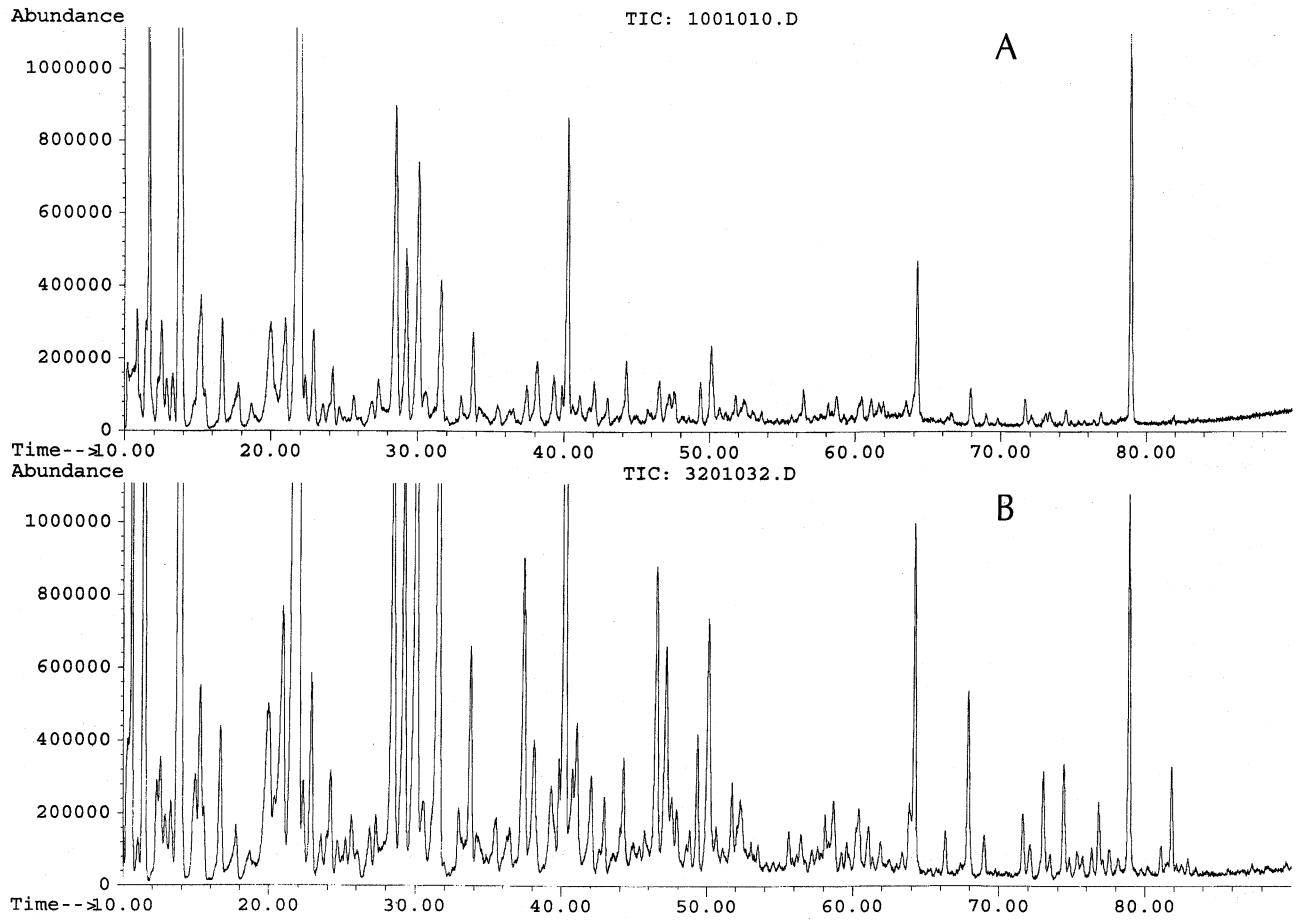

Figure 7. Vapor phase profiles of EXP-C (A) and FFLT-1 (B).

the IS was used in calculating the amounts of all peaks in the chromatograms, hence accurate integration of the IS was especially important. To increase the accuracy of determining the area of the IS, an extracted ion chromatogram of *m/z* 136, specific for the IS, was used. The IS peaks in the extracted ion chromatograms were integrated, and the IS areas in the total ion chromatograms were determined by dividing those areas by 0.31, which was the fraction of *m/z* 136 in the mass spectrum of $^2H_7$-quinoline as obtained from a blank sample. A comparison of the IS peak in the total ion and extracted ion modes (from an enlarged portion of a mainstream smoke particulate phase chromatogram) is shown in Figure 1.

Combining contents of vapor phase traps

Impinger tube contents were combined to simplify data collection and reduction and to minimize errors associated with integration. Arguably, the contents of the four vapor phase traps could have been analyzed separately. Although this would lead to reduced chromatographic interference for some compounds, it would also cause problems with detection limits for some compounds (those which would be distributed across traps at concentrations less than the 0.5 µg/cigarette integration threshold). Analysis of separate traps would also necessitate the identification (or at least the similarity) of peaks across all chromatograms. Accurate integration is a primary source of error in the determination of semiquantitative measures. Requiring the addition of integration results for a compound present in multiple chromatograms would increase the effects of this error.

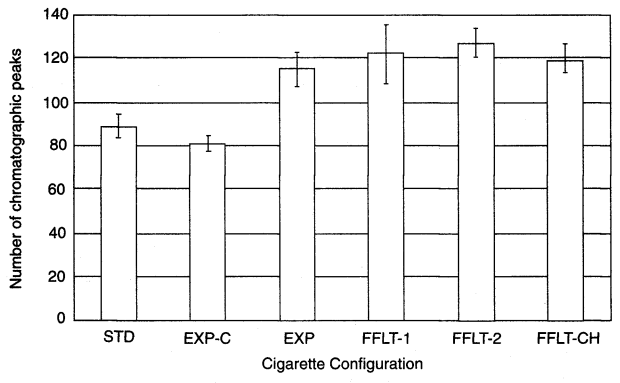

Figure 8. Comparison of the number of chromatographic peaks for EXP-C and control products.

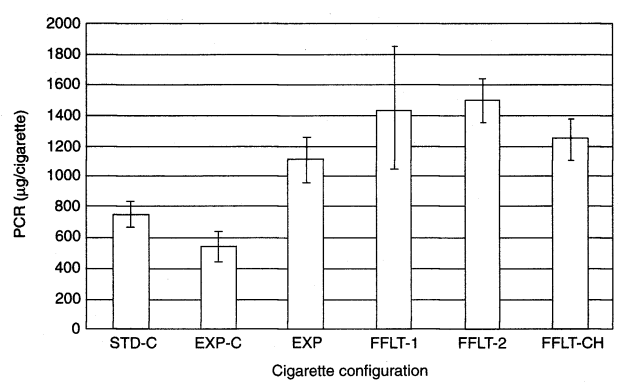

Figure 9. Comparison of PCRs for EXP-C and control products.

Major components in particulate phase profiles

As shown in Figure 2, there are five peaks that tend to dominate the particulate phase profiles: acetic acid, propylene glycol, nicotine, triacetin, and glycerol. When comparing particulate phase samples, it is useful to analyze the data excluding these compounds because they tend to mask the contributions of the many smaller peaks in the chromatograms.

Validation of semiquantitative measures

Mainstream smoke from a series of four Kentucky reference cigarettes was profiled to ensure that increasing TPMs correlated well with increases in measured chromatographic response. The series included 1R5F, 1R4F, 1R3F, and 2R1F cigarettes with measured TPMs (milligrams per cigarette based on the FTC puffing regimen) of 2.37, 11.08, 17.20, and 27.67, respectively. The linear relationship (regression coefficient $R^2 = 0.996$) between measured TPM and particulate phase PCR is shown in Figure 3.

TOB-HT study

This study focused on a cigarette which primarily heated, rather than burned, tobacco (TOB-HT). A commercial "ultra low tar" cigarette (COM-ULT) and a research cigarette (1R4F) served as bases for comparisons. All reported measures were based on the results from six replicate determinations. To show detail and facilitate comparisons, all chromatograms shown were scaled to the IS (vapor phase, 76.1 min; particulate phase, 46.7 min) for direct visual comparison of the minor peaks on a per cigarette basis, which caused the largest peaks to go off the scale.

Vapor phase profiles of TOB-HT, COM-ULT, and 1R4F are shown in Figure 4. Upon visual examination of these chromatograms, it is readily apparent that the TOB-HT chromatogram (Figure 4A) is less complex in terms of number and response of peaks than the COM-ULT (Figure 4B) and 1R4F (Figure 4C) chromatograms. These observations are supported by the semiquantitative measures of the number of peaks, PCR, and TCR (Table II).

Like the vapor phase profiles, the particulate phase profile for TOB-HT (Figure 5A) is far less complex than the profiles of COM-ULT (Figure 5B) and 1R4F (Figure 5C). Semiquantitative measures for particulate phase data are summarized in Table III.

The mainstream smoke from the test cigarettes can be further examined in terms of reductions in some of the relevant semi-

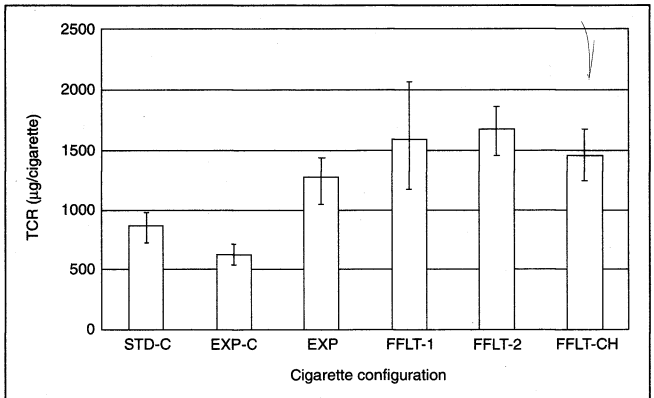

Figure 10. Comparison of TCRs for EXP-C and control products.

quantitative measures. Compared to 1R4F, reductions in the number of peaks (vapor phase, 74%; particulate phase, 95%) and TCR (vapor phase, 89%; particulate phase, 70%; particulate phase excluding major components, 93%) were seen for the TOB-HT mainstream cigarette smoke. These reductions were much greater than would be expected, based on relative TPM.

EXP-C study

In the EXP-C study, a cigarette that contained an experimental tobacco blend and a new carbon filter was compared with two commercial leading "light" cigarettes (FFLT-1 and FFLT-2), a commercial charcoal filter "light" cigarette (FFLT-CH), a cigarette that contained the new carbon filter but had a standard U.S. tobacco blend (STD-C), and a cigarette that contained the experimental tobacco blend but had a standard cellulose acetate filter (EXP).

A comparison of the mainstream smoke from EXP-C (Figure 6A) and FFLT-2 (Figure 6B) showed that, although the particulate phase fractions were similar in complexity, the vapor phase profile of EXP-C (Figure 7A) appeared to have fewer peaks and lower peak responses than the FFLT-2 (Figure 7B) profile. As in the TOB-HT study, all chromatograms were scaled to the IS, and all data were based on six replicate experiments.

As shown in Table IV, the mainstream smoke particulate phase semiquantitative measures for EXP-C were comparable with those of the other cigarette configurations. In contrast, relative to the three commercial products, the vapor phase of EXP-C had a reduced number of chromatographic peaks (32–36%) (Figure 8), reduced PCR (56–64%) (Figure 9), and reduced TCR (57–62%) (Figure 10). Figures 8–10 also show that these reductions were primarily due to the effects of the new carbon filter (STD-C configuration).

Scope and limitations

As currently practiced in our laboratories, profiling is confined to the fraction of smoke that is sufficiently volatile for GC analysis. Components trapped in the vapor phase meet this volatility requirement. Thermogravimetric analyses of TPM from tobacco-burning cigarettes show that it is approximately 60% volatile at temperatures emulating GC analyses (220–230°C) (11). At these temperatures, thermogravimetric analyses of TPM from cigarettes that primarily heat tobacco indicate that approximately 95% of the TPM is volatile (M.F. Borgerding, J.A. Bodnar, H.L. Chung, P.P. Mangan, C.C. Morrison, C.H. Risner, J.C. Rogers, D.F. Simmons, M.S. Uhrig, F.N. Wendelboe, D.E. Wingate, and L.S. Winkler. Submitted for publication in *Food Chem. Toxicol.*).

In determining chromatographic responses, all calculations were based on the assumption that all components have response factors equivalent to that of the IS. Since this is clearly not the case, profiling is limited to comparative rather than absolute measures.

Conclusion

Chromatographic profiling has proven to be a useful tool in comparing the chemical complexity of different matrices. In addition to the standard semiquantitative information provided, the use of an information-rich detector, such as a mass spectrometer, enables the analyst to identify specific compounds. Profiling was effective in showing that both the vapor phase and particulate phase fractions of mainstream cigarette smoke from TOB-HT were greatly reduced in complexity compared with control products. Profiling also showed that, although the particulate phase fraction of mainstream cigarette smoke from EXP-C was similar to the control products, the vapor phase fraction of EXP-C contained reduced numbers of peaks, reduced PCR, and reduced TCR, as compared with the control products. Profiling information is seldom used alone but usually as a complementary part of larger product evaluation strategies (M.F. Borgerding, J.A. Bodnar, H.L. Chung, P.P. Mangan, C.C. Morrison, C.H. Risner, J.C. Rogers, D.F. Simmons, M.S. Uhrig, F.N. Wendelboe, D.E. Wingate, and L.S. Winkler. Submitted for publication in *Food Chem. Toxicol.*).

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References

1. K. Grob. Gas chromatography of cigarette smoke, part III. Separation of the overlap region of gas and particulate phase by capillary columns. *J. Gas Chromatogr.* **3:** 52–56 (1965).
2. K. Grob. The gas chromatographic separation of cigarette smoke, part 2. Refined separation using capillary columns. *Beitr Tabakforsch.* **9:** 315–23 (1962).
3. K. Grob. Gas chromatography of cigarette smoke, part 4. Identification with the aid of a mass spectrometer. *Beitr Tabakforsch.* **3:** 403–408 (1966).
4. K. Grob. Gas chromatography of cigarette smoke, part I. A method for the routine analysis of the gas-vapour phase. *Beitr Tabakforsch.* **7:** 285–90 (1962).
5. M.F. Dube and C.R. Green. Methods of collection of smoke for analytical purposes. *Recent Adv. Tob. Sci.* **8:** 42–102 (1982).
6. R.A.W. Johnstone and J.R. Plimmer. The chemical constituents of tobacco and tobacco smoke. *Chem. Rev.* **59:** 885–936 (1959).
7. R.L. Stedman. The chemical composition of tobacco and tobacco smoke. *Chem. Rev.* **68:** 153–207 (1968).
8. G.D. Byrd, K.W. Fowler, R.D. Hicks, M.E. Lovette, and M.F. Borgerding. Abstract #54: Mainstream vapor phase smoke comparison of a 1R4F Kentucky reference cigarette and a new cigarette that heats rather than burns tobacco. 42nd Tobacco Chemists' Research Conference (1988).
9. J.N. Diana and A. Vaught. *Research Cigarettes.* University of Kentucky Printing Services, Lexington, KY, 1990.
10. Federal Trade Commission. Cigarettes: Testing for "tar" and nicotine content—statements of consideration. *Federal Register.* **32:** 11178 (1967).
11. M.F. Borgerding, R.D. Hicks, J.E. Bodnar, D.M. Riggs, E.J. Nanni, G.W. Fulp, Jr., W.C. Hamlin, Jr., and J.A. Giles. Cigarette smoke composition. Part 1. Limitations of FTC method when applied to cigarettes that heat instead of burn tobacco. *J. Assoc. Off. Anal. Chem.* **73:** 605–609 (1990).

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