Quantitative Analysis of Acetates in Cigarette Tobacco using Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry

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Abstract

A method incorporating solid-phase microextraction (SPME) and gas chromatography–mass spectrometry for the headspace analysis of selected volatile organic compounds present in cigarette tobacco is developed and evaluated. Quantitative information on methyl, ethyl, *n*-propyl, isopropyl, isopropenyl, vinyl, and butyl acetates present in 29 different flavor variants (full, light, and ultra-light) of the top ten selling brands in the United States is presented. The concentrations of the various acetate analytes range from the low nanaogram to microgram levels per cigarette. Clear differences are observed in the concentrations of various acetates when comparing the levels in brands from different manufacturers. The SPME technique provides a method that allows high sample throughput, requires little sample preparation, and yields useful analytical information. High precision is obtained on multiple measurements of cigarettes from an individual pack, but lower precision levels are observed in general when comparing results obtained on the analysis of cigarettes from different packs of the same brand. The higher pack-to-pack variations may be due in part to product aging with a proportionate amount of evaporative loss of the relatively volatile acetates.

Introduction

Cigarette smoking has dramatic public health consequences and is the leading preventable cause of death in the United States (1). Numerous reports on the components of tobacco smoke have identified the presence of at least 4000 different chemical substances (2–4), many of which are know to have harmful properties. Most of the components in smoke are the products of combustion or pyrolysis that form during the smoking process. However, some chemicals, such as nicotine and menthol, are present in both the unburned cigarette and in the smoke. Not yet fully addressed are several questions about the chemicals present in processed tobacco and in mainstream smoke (smoke that passes through the cigarette during the smoking process), such as what fraction of chemicals initially present in tobacco enters the smoke stream and does the presence of such chemicals induce interactions that potentially alter the composition or concentration of other chemical species present in the smoke. To begin to address such questions, a comprehensive and systematic survey of the top ten brands of cigarettes (5) was initiated in order to obtain quantitative information about selected volatile and semivolatile organic chemicals initially present in cigarette tobacco.

To facilitate this process, an analytical method incorporating a solid-phase microextraction (SPME) technique (6,7) for the headspace analysis of tobacco was developed and evaluated. The SPME technique is ideally suited for this type of analysis, because (*a*) the SPME fiber's stationary phase serves both as a sample collection and preconcentration element, (*b*) low detection limits with wide concentration linearity are achieved, and (*c*) the use of solvents required for conventional purge-and-trap headspace analysis are eliminated. The SPME technique has proven extremely powerful when used either for direct extraction of analytes from liquids or for headspace analysis. Applications using SPME cover extremely diverse areas, such as analyses of air and water pollution (8,9), soil (10), volatile compounds in biological fluids (11), and wine analysis (12). For tobacco research, the utility of SPME has been demonstrated for the analysis of various flavor additives (13), phenolic compounds in cigarette smoke condensate (14), and various alkaloids present in tobacco (15).

Our laboratory has extensive experience in analyzing volatile compounds mainly using the purge and trap technique (16–18), but excessive sample handling, sample carryover concerns, growing disposal costs of solvents, and environmental concerns over the disposal methods have made alternative analytical technologies such as SPME attractive. As new technologies become available, they must be evaluated and well characterized before they are widely used. Therefore, SPME was evaluated as a method for performing quantitative analysis of selected volatile organic compounds (VOCs) present in

commercial cigarette tobacco using gas chromatography–mass spectrometry (GC–MS).

The present work demonstrates several positive aspects in using SPME to analyze tobacco. Advantages of the method include (*a*) rapid analysis (30 min for SPME–GC–MS analysis of an individual cigarette), (*b*) high throughput using an autosampler for unattended operation, (*c*) simplified sample work-up, and (*d*) the absence of solvent waste. In this proof-of-concept report, 7 acetate analytes were selected as target molecules, because they are often used as flavorants that impart a fruity or floral odor (19), exhibit a wide range of concentrations depending on brand, and are relatively benign in comparison with a variety of other chemicals present in processed tobacco. Quantitative analysis was performed on 29 individual flavor variants of the top ten selling cigarette brands including full flavor (> approximately 15 mg tar), light (approximately 5–15 mg tar), and ultra-light (< approximately 5 mg tar) cigarettes for levels of methyl, ethyl, *n*-propyl, isopropyl, isopropenyl, vinyl, and butyl acetate. Presented are the results of the 29 individual cigarette types and analytical figures of merit for the SPME–GC–MS technique, including linearity, limit of detection (LOD), and reproducibility.

Experimental

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.

Materials

Chemical reagents were purchased from several commercial vendors. Methyl, ethyl, *n*-propyl, isopropyl, isopropenyl, vinyl, and butyl acetates used as standards in this study were purchased from Aldrich Chemical Company (Milwaukee, WI). The primary labeled internal standard, ^{13}C-ethyl acetate, was purchased from Cambridge Isotope Laboratories (Andover, MA). A second internal standard, tetrahydrofuran (THF, high-performance liquid chromatography grade), and methanol (purge-and-trap grade) used as a solvent for initial dilutions of the standards, were purchased from Burdick and Jackson (Muskegon, MI). In order to obtain water relatively free of volatile organic species, rural well water (used in the final dilution steps) was purified by refluxing while purging it with helium for a minimum of 6 h and then collecting it via distillation. The water was dispensed in 100-mL bottles, sealed with Teflon-lined caps, and placed in a desiccator until needed. Full-scan GC–MS analysis showed that residual contamination of the water was below the detection limits of the current study. The purity of the other chemicals was also established with GC–MS analysis.

Full flavor, light, and ultra-light cigarettes from the top ten leading brands were randomly purchased from a variety of retail locations in the metropolitan Atlanta area. After purchase, the cigarettes were promptly transferred into 40-mL screw-top glass vials that were sealed using Teflon-lined caps and stored at –70°C. Research-grade cigarettes (1R5F) purchased from the University of Kentucky (Lexington, KY) were used to generate a blank tobacco matrix as described in the following paragraphs.

Preparation of materials

Tobacco from ten packs of 1R5F cigarettes was extracted from the paper wrapper and filter and placed in a gas-washing bottle (Kimble Glass, Vineland, NJ). The gas-washing bottle was used to purge the tobacco of VOCs. The tobacco was inserted and rested on the sintered glass surface. A helium cylinder was connected to the gas port below the sintered glass. During a 48-h period, helium gas (approximately 0.5 L/min) flowed through the tobacco to purge it of volatile components. After purging with helium, the tobacco was removed and placed in an evacuated container that was maintained at approximately 1×10^{-2} torr for 10 days to remove any residual VOCs. Before the container was opened and the tobacco removed, the chamber was repressurized to atmospheric pressure using helium gas, and the resulting tobacco was transferred in 1.5-g increments to a series of 10-mL vials that were sealed with Teflon-lined caps and stored at –70°C.

All glassware, including volumetric flasks, 10-mL serum vials used for SPME headspace analysis, 10-mL vials used to store the blank tobacco, and 40-mL cigarette storage vials, was cleaned, rinsed with methanol, and baked in an evacuated oven at 120°C for a minimum of 24 h to remove any residual volatile contamination. The glassware was cooled to room temperature under vacuum and purged with nitrogen gas during its removal from the oven. The glassware was then rapidly sealed and stored until needed. The serum vials used for SPME analysis were crimped tightly shut using Teflon-lined septa (25 × 20-mm diameter) and stored in a desiccator.

In the preparation of stock solutions, the transfer of chemical reagents at the microliter level was done using positive displacement pipettes. To minimize contamination, the glass pipette tips were used once and then discarded. Analyte standards were prepared by successive dilutions in methanol after weighing the neat compounds to the nearest 0.1 mg. Similarly, stock solutions that contained ^{13}C-ethyl acetate and THF were prepared for use as an internal standard.

Due to the volatility of the chemicals under investigation, stock solutions of the standards and the internal standard with intermediate concentrations of the acetates under study were sealed in a series of 1-mL glass ampules and stored at 4°C. The contents of these vials were diluted in water to the desired final concentrations. Multiple aliquots from three different stock solutions of the analytes under investigation with concentrations of 10, 1, and 0.1 ng/µL were used to generate the calibration curves. The final concentration of the internal standard solution was 1 ng/µL. The resulting solutions were stored at 4°C and used for a maximum of 3 days to minimize variations in concentration due to evaporation. The concentrations of the standards were chosen in an attempt to span the entire concentration range of analytes in the tobacco in order to provide good accuracy. The range of concentration that can be

measured is somewhat limited in isotope dilution mass spectrometry (IDMS) due to the mass spectral peak overlap between the analyte and the labeled analogue (20).

After separation from the paper wrapping and filter plug, the tobacco from an individual cigarette was placed in a 10-mL serum vial. A 50-µL aliquot of the internal standard solution and 250 µL of water was added before a teflon-lined septa was crimped on the top of the vial to ensure a tight seal. The contents of the vial were tumbled on a rotary mixer for 1 h to ensure that thorough mixing had occured and to allow time for the various gaseous components to establish equilibrium concentration in the headspace. To generate standard curves and sample blanks, 0.5 g of the purged 1R5F tobacco was placed in a 10-mL serum vial, and a 50-µL aliquot of the internal standard solution, 250 µL of water, and in the case of the standards, either a 25-, 50-, or 100-µL aliquot of the appropriate stock solution was added.

Instrumentation

A series of twelve 10-mL serum vials were placed in the sample tray of a Varian (Sugarland, TX) 8200 autosampler model 8200, and the headspace above the tobacco in each vial was sequentially sampled with a 75-µm Carboxen-PDMS fiber (Supelco, Bellefonte, PA). The autosampler holds a maximum of 12 vials, and typically, each sample run consisted of one 0.5-g 1R5F blank sample, one analyte standard that corresponded to a point on the calibration curve and served as a quality control check, and ten cigarette tobacco samples. Typically, all twelve samples were analyzed in triplicate. After a 5-min exposure to the headspace above the tobacco sample, the fiber was introduced into the heated inlet of a Hewlett-Packard (Palo Alto, CA) 6890 GC. To prevent inadvertent adsorption of organic vapors that are occasionally present at low concentrations in our laboratory, the time the fiber was outside a SPME vial or the GC inlet was minimized. The SPME sampling assembly was operated such that the fiber was either absorbing sample vapors in the headspace of a serum vial or kept in the inlet of the GC until the next analysis was ready.

The injection inlet, which was operated in splitless mode and maintained at 285°C, used a narrow-bore (75 µm) inlet liner. The relatively high inlet temperature was necessary in order to eliminate sample carryover between samples for certain chemicals present in the tobacco, such as propylene glycol, acetic acid, and menthol. The chromatograph was equipped with a 1-m fused-silica precolumn and a J&W Scientific (Folsom, CA) DB-624 column with a 320-µm diameter and a 1.8-µm film thickness. A constant flow of 3.0 mL/min was maintained through the column using helium as a carrier gas. The following temperature program was used: hold at 35°C for 3.5 min, ramp to 190°C at 12°C/min, and hold at 190°C for 5 min. The GC–MS transfer interface was maintained at 230°C (below the 260°C limit of the DB-624 column), and no sample carryover was observed with the elevated inlet temperature (285°C).

A Hewlett-Packard model 5973 MS was used for data acquisition. Instrument tuning and mass calibration were checked daily using perfluorotributylamine. Full-scan mass spectra were acquired covering a mass range of 29 to 200 amu at a rate of 4.19 scans/s. Table I shows the quantitation masses, confirmation masses, and retention times for each of the analytes and internal standards. All mass spectral results were manually evaluated for proper integration limits, correct baseline determination, interferences, and confirmatory masses. After the reconstructed ion chromatogram had

Table I. Quantitation Masses, Confirmation Masses, and Retention Times for the Analytes Studied

Compound	Quantitation mass (amu)	Confirmation mass (amu)	Retention time (min)
Methyl acetate	74	59	2.97
Ethyl acetate	61	70	4.96
n-Propyl acetate	61	73	7.26
Isopropyl acetate	61	87	6.09
Isopropenyl acetate	58	72	6.51
Vinyl acetate	86	44	4.14
Butyl acetate	73	56	9.22
^{13}C-ethyl acetate	62	71	4.96
THF	72	42	5.16

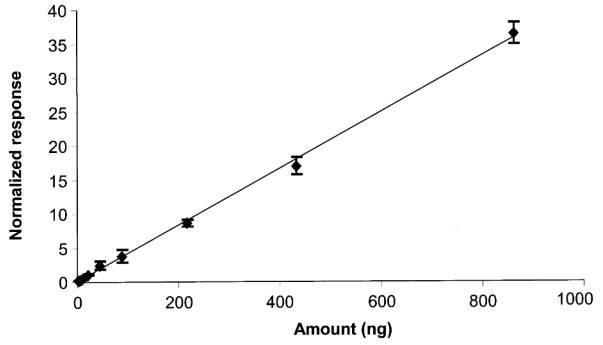

Figure 1. Calibration curve for *n*-propyl acetate is typical of that obtained for all analytes. A minimum of eight measurements was obtained for each point, and the error bars reflect the 95% confidence limits.

Table II. Analytical Figures of Merit Obtained for the Selected Acetate Analytes

Compound	Slope (value ± standard error)	*y*-intercept (value ± standard error)	R^2	Recovery % ± RSD*	LOD (ng/cigarette)
Methyl acetate	0.0065 ± 0.0001	0.147 ± 0.076	0.999	78 ± 3.2	2.2
Ethyl acetate	0.0232 ± 0.0003	0.111 ± 0.108	0.999	95 ± 4.0	2.5
Vinyl acetate	0.0155 ± 0.0007	0.004 ± 0.138	0.989	111 ± 7.9	1.1
Isopropyl acetate	0.0371 ± 0.0005	0.121 ± 0.380	0.999	106 ± 3.5	2.1
n-Propyl acetate	0.0416 ± 0.0006	0.041 ± 0.519	0.998	108 ± 4.5	2.9
Isopropenyl acetate	0.0276 ± 0.0010	0.102 ± 0.207	0.975	66 ± 8.5	2.7
Butyl acetate	0.0117 ± 0.0002	0.012 ± 0.072	0.998	111 ± 6.6	1.1

* LOD, limit of detection.
† RSD, relative standard deviation.

been checked, the tabulated peak area data were exported to a spreadsheet program for further analysis. Because of the overlap of the isotope profiles of native ethyl acetate and the ^{13}C-ethyl acetate label, corrections were made to quantitation peak areas according to previously described methods (20,21). To obtain good accuracy, all samples were prepared daily, and a minimum of three independent measurements were made in triplicate over a period of several days.

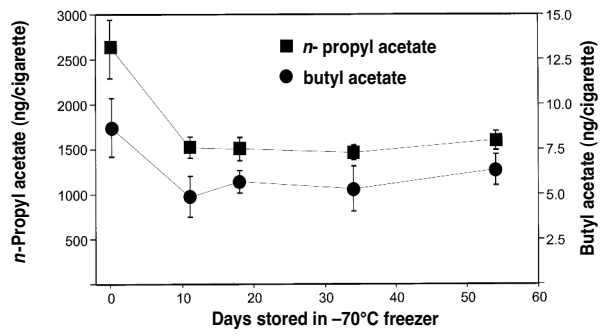

Figure 2. The levels of *n*-propyl and butyl acetate detected in cigarettes after removal from their original packaging and storage in glass vials at –40°C.

Table III. Comparison of the Precision Obtained for Multiple Analyses over a One-Day Period and a Multiple-Day Period

	Short term (1 day, n = 3)		Long term (43 days, n = 8)	
Acetate	Amount (ng/cigarette)	%RSD*	Amount (ng/cigarette)	%RSD*
Ethyl acetate	21	2	9	35
Methyl acetate	216	6	83	75
Isopropyl acetate	58	4	70	4
n-Propyl acetate	778	3	888	4
Butyl acetate	3	3	5	14
	Average RSD = 3.6%		Average RSD = 26.4%	

* %RSD, percent relative standard deviation.

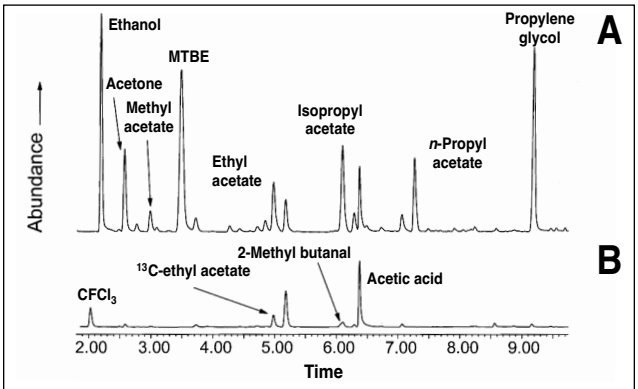

Figure 3. Comparison of the total ion chromatogram. of a typical cigarette brand having intermediate levels of acetate analytes (A) and of the purged 1R5F used as an acetate-free matrix (B).

Results and Discussion

Calibration curves for the seven acetate analytes were generated using an 8- or 9-point curve that spanned the nanogram to microgram range. A typical calibration curve is shown for *n*-propyl acetate in Figure 1. An average of 8 measurements was obtained for each point in the calibration curve, and the error limits were calculated using a 95% confidence interval. These data were obtained by (*a*) adding known amounts of the standard to 0.5 g of the purged 1R5F tobacco, (*b*) measuring the peak area from the reconstructed ion chromatogram, (*c*) correcting for overlap with the internal standard (and vice versa), and (*d*) dividing by the area of the internal standard. The ^{13}C-labeled ethyl acetate was used as an internal standard for all analytes except methyl acetate. For methyl acetate, THF was used as the internal standard because it provided a more consistent normalized response than the ^{13}C-labeled ethyl acetate. Table II shows the slope, *y*-intercept, correlation coefficient (R^2), LOD, and percent recovery obtained for each analyte.

The R^2 values were all greater than 0.95, with an average R^2 of 0.990 for all the analytes. A linear response between the analyte's normalized peak area and the amount of added

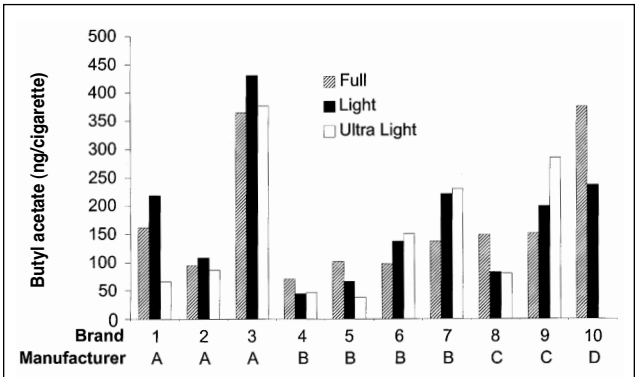

Figure 4. Methyl acetate levels detected in 29 individual variants (full flavor, light, and ultra-light) of the top ten selling brands. A minimum of three independent measurements was obtained for each brand variant. Average RSD for all methyl acetate measurements was 28%.

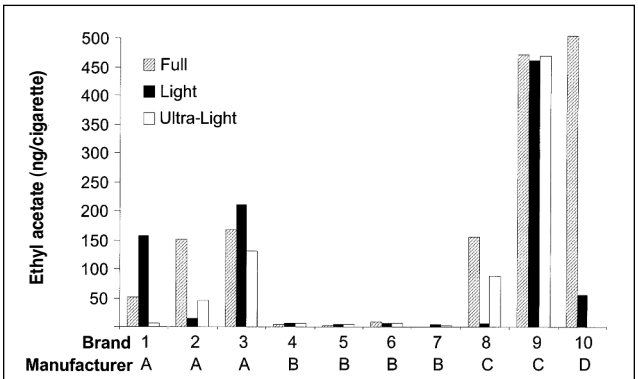

Figure 5. Ethyl acetate levels detected in 29 individual variants (full flavor, light, and ultra-light) of the top ten selling brands. A minimum of three independent measurements was obtained for each brand variant. Average RSD for ethyl acetate measurements was 12% for concentrations above 10 ng and 30–60% for levels below 10 ng.

standard was observed for more than three orders of magnitude. With the lowest point on the curve being near the detection limit, a near zero value of the *y*-intercept for each calibration curve was calculated using a least squares fit. The low value of the *y*-intercept served both as a check that little or no acetate remained in the blank tobacco matrix and indicated that quantitation at the lower concentration levels should provide reliable results.

The LOD values were determined from a plot of the standard deviation of the calculated concentration versus the spiked concentration (22). The *y*-intercept of the least square fit line provided a good estimate of the standard deviation at "zero" concentration (s_0), with $3s_0$ being the calculated detection limit. For this particular study, we choose the higher of either $3s_0$ or the lowest point on the calibration curve as a reliable and conservative estimate of the LOD value. The LOD values ranged from 1 to 3 ng, and these values represent the minimum material present per cigarette that would produce a "detectable" signal for this method. Obviously, operating the MS in selected ion monitoring (SIM) mode instead of full-scan mode would allow for a substantial further reduction in the detection limits.

A recovery study was performed by spiking a solution containing approximately 45 ng each of seven analytes on five different 0.5-g samples of the 1R5F blank matrix and measuring the relative response factors for each sample. Five 0.5-g 1R5F tobacco blanks were also analyzed to check for the presence of acetates in the blank material. The data obtained for the spiked tobacco were averaged and background corrected by subtracting out any contributions from analytes detected in the blank tobacco. The only analyte observed in the tobacco blanks that was above trace levels was a very small signal for methyl acetate. The percent recovery ranged from 66 to 111% with an average recovery of 96% for all seven analytes. It is not known why isopropenyl acetate had only 66% recovery.

During the initial phase of this investigation, new and opened packages of cigarettes were placed in plastic ziplock bags and stored at –70°C. We noticed that the analyte levels decreased steadily over the course of several weeks. This finding was not totally unexpected, given the volatile nature of the analytes being studied, but to ensure sample integrity during the course of this investigation, an alternative storage method was evaluated by measuring the acetate levels of cigarettes from the same pack over an 8-week period. The acetate levels in cigarettes from a freshly opened pack were analyzed immediately after the pack was opened, while the remaining cigarettes were placed in a series of 40-mL glass vials (4 cigarettes per vial) and stored at –70°C, as previously described. Cigarettes were removed and analyzed as needed over a period of 54 days. Figure 2 presents the storage results for *n*-propyl and butyl acetate, the two acetates of highest and lowest concentration in this brand of cigarettes.

The concentration of the volatile acetates detected in cigarettes stored in glass vials remained fairly constant, a finding that validates the use of this storage method. The calculated relative standard deviation (RSD) for the propyl- and butyl acetate data shown in Figure 2 are 8.5% and 17.8%, respectively. However, as shown in Figure 2, there was an approximate 33% drop in the acetate concentration of the stored cigarettes in comparison with the concentration in cigarettes from a freshly opened pack. Presumably, the relatively volatile acetates present in the cigarettes are free to migrate through the tobacco and the inside surfaces of the packing materials and establish new equilibrium concentration levels. Once an individual pack is opened, loss of VOCs such as the acetates under investigation occurs. Removing cigarettes from their

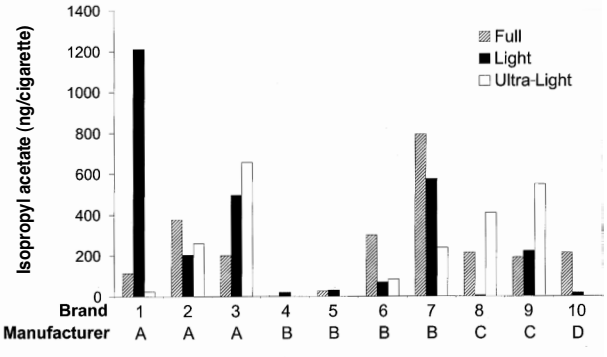

Figure 6. Isopropyl acetate levels detected in 29 individual variants (full flavor, light, and ultra-light) of the top ten selling brands. A minimum of three independent measurements was obtained for each brand variant. Average RSD for all isopropyl acetate measurements was 8.6%.

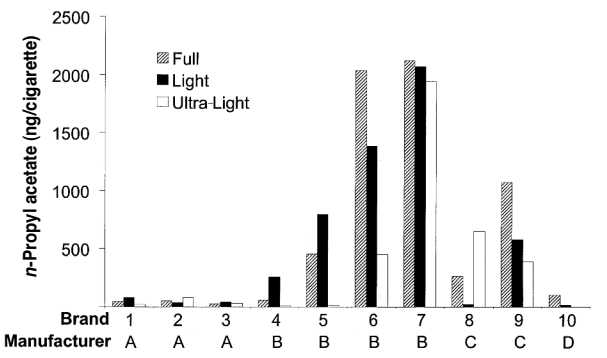

Figure 7. *n*-Propyl acetate levels detected in 29 individual variants (full flavor, light, and ultra-light) of the top ten selling brands. A minimum of three independent measurements was obtained for each brand variant. Average RSD for all *n*-propyl acetate measurements was 7%.

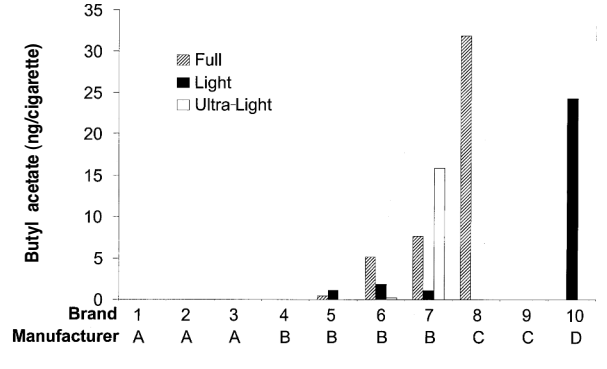

Figure 8. Butyl acetate levels detected in 29 individual variants (full flavor, light, and ultra-light) of the top ten selling brands. A minimum of three independent measurements was obtained for each brand variant. Average RSD for all butyl acetate measurements was 30%.

original packs and storaging them in glass vials results in a decrease in acetate concentrations as a new equilibrium is established in the acetate-free glass containers.

The precision of the SPME method was observed to vary inversely with acetate concentration. Typically, lower acetate concentration values had significantly higher RSDs. In addition, as would be expected, higher precision is obtained for a series of multiple acetate measurements obtained in a single day (short time interval) than for measurements recorded over several days (long time interval). Table III sumarizes the comparison of a series of multiple measurements made on full-flavor brand 6 on a single day with those obtained over a period of 43 days. The short-term data represent the results of analyzing three individual cigarettes in triplicate to produce an average RSD of 3.5%. The low RSD indicates that good precision was obtained. For the long-term study, eight individual cigarettes were removed sequentially from their respective glass vials (maintained at –70°C) and analyzed over a 43-day period. The long-term analysis produced an average RSD of 26.4%, and the resultant loss in precision is hopefully offset, at least in part, by a gain in accuracy. The differences in precision between the short-term and long-term studies may be attributed to one or more factors including evaporative losses of the more volatile components, slight differences in the preparation of the internal standard, differences in the acetate concentrations of individual cigarettes, and natural chemical reactions occurring in the tobacco.

Sample collection and introduction into the GC inlet via the SPME technique not only produces good precision and reproducibility, but also yields good chromatographic behavior. A comparison of the chromatograms obtained from a typical cigarette sample and the blank 1R5F tobacco is shown in Figure 3. The purging procedure for the 1R5F tobacco previously described effectively removes nearly all of the volatile acetates, although other compounds can be detected, as seen in Figure 3B. The top chromatogram (Figure 3A) shows good peak separation and peak shape for a typical sample that contained moderate levels of the acetate analytes and relatively high concentrations of other naturally occurring and added volatile compounds presumably used as solvents, flavors, and humectants.

Of the ten cigarette brands analyzed, nine brands have a full-flavor, light, and ultra-light product, whereas one brand has only a full-flavor and light variant. Thus, we analyzed 29 different variants, or "flavors", of cigarettes. The measured concentrations for two of the seven acetates (vinyl- and isopropenyl acetates) were below the detection criteria for all brands and are reported as nondetectable using the current approach. It was also found that the concentrations of the remaining five acetates (methyl, ethyl, *n*-propyl, isopropyl, and butyl acetate) had brand-dependent concentrations ranging from the detection limit (1–3 ng) to approximately 3 µg. For several cigarette brands, the detected concentrations of one or more analyte were outside the range of the calibration curve. In these cases, the cigarettes were analyzed using approximately 33% (w) of the tobacco from a single cigarette to achieve levels within the calibration range. These values were then scaled to a per-cigarette value for reporting.

Figures 4–8 summarize the results for the analyses of methyl, ethyl, *n*-propyl, isopropyl, and butyl acetates, respectively, and the individual cigarette brands are grouped according to manufacturer. In reviewing these data, several interesting trends were noted. Methyl acetate levels (Figure 4) range from 25 to 450 ng per cigarette and exhibit no clear differences in concentration for either flavor variant or manufacturer. However, as seen in Figures 5, 7, and 8, relatively low levels of ethyl acetate and much higher levels of both *n*-propyl and butyl acetate were detected in manufacturer B's products in comparison with those of manufacturer A. In addition, although cigarettes from manufacturer A had very low levels of *n*-propyl acetate, they had relatively high levels of isopropyl acetate; the opposite was true for cigarettes from manufacturer B (Figures 6 and 7). Levels of most acetates in

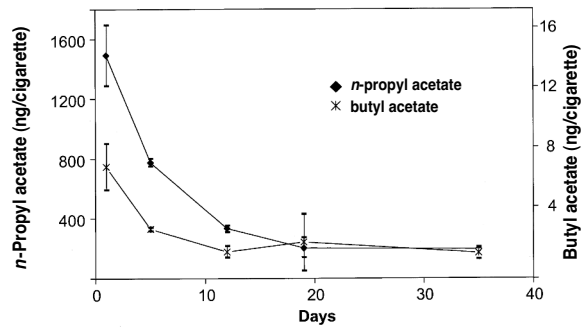

Figure 9. Levels of *n*-propyl and butyl acetates detected in a cigarette from a single carton with the individual packs opened on different days.

Table IV. Pack-to-Pack Comparison of Acetate Analyte Concentration Level Detected in Different Packs of Brand 6

	Pack 1		Pack 2		Pack 3		Pack 4		Pack 5	
	Mean* (ng/cigarette)	RSD (%)	Mean (ng/cigarette)	RSD (%)	Mean (ng/cigarette)	RSD (%)	Mean (ng/cigarette)	RSD (%)	Mean (ng/cigarette)	RSD (%)
Ethyl acetate	7	60	8	65	8	61	13	44	3	20
Methyl acetate	93	35	70	23	87	12	83	18	142	30
Isopropyl acetate	78	26	222	9	36	10	50	31	348	3
n-Propyl acetate	848	28	1352	10	387	9	1125	33	2276	10
Butyl acetate	1	26	1	12	2	18	6	38	5	21

* Measurement obtained for 5 cigarettes from each pack.

cigarettes from manufacturers C and D generally fell somewhere in between the levels measured in cigarettes from manufactures A and B, except for higher levels of ethyl and butyl acetates in several C and D products. The difference in acetate concentrations detected in the various cigarettes could have resulted from differences in tobacco blends, manufacturing processes, the cigarettes' storage, or the use of various flavorants or additives.

The cigarette-to-cigarette reproducibility from a single pack of cigarettes provided reasonable precision levels. However, the question arises as to whether the fluctuations in concentrations levels for selected acetate analytes in various manufacturer's brands are related to differences in processing, handling, or storage. In order to further investigate this question, acetate levels in different packs of the same brand were compared. To obtain additional quantitative information and pack-to-pack precision values, five individual packs of brand 6 (full flavor) with differing lot numbers were randomly purchased over the course of this investigation and analyzed. Table IV shows the quantitative results for the acetate levels in five different packs of brand 6. The comparison of the acetate data obtained from the five packs reveals an approximate fourfold variation in the analyte concentrations. The average RSD for all analytes in the five packs was 26%, with the largest RSD observed for the analytes having the lowest concentrations. One possible explanation for the high pack-to-pack variation could be related to varying losses of the volatile components due to differences in the ages of the products.

To test this hypothesis, we purchased a carton (ten packs) of brand 6 (full flavor, all packs having identical lot numbers) and then stored it at 72°F and 60% relative humidity in an environmental control chamber. The cigarette packs were removed from the original paper carton when stored in the environmental chamber, but the original wrappings of the individual packs remained intact. The individual packs were removed and analyzed for a period of several weeks. For each analysis, a new pack was removed from the environmental chamber; the outer cellophane and foil packaging was opened, and five cigarettes were removed and analyzed. The levels of acetates were observed to decrease by a factor of 7 over the 35 days of the experiment (Figure 9). Kinetic analysis of the time-dependent data shown in Figure 9 indicates that the loss of acetate from a sealed pack can be modeled fairly well using a second-order rate expression. Such behavior may account for the initial rapid decline in acetate levels, followed by a much slower loss, yielding fairly steady concentration levels over longer periods. The calculated half-life for loss of acetates from an individually sealed pack of brand 6 is approximately 8 days. Therefore, the higher pack-to-pack fluctuations of the acetate levels could be consistent with a 2- to 3-week interval when products remained on vendors' shelves.

Conclusion

The SPME technique has proven useful in identifying and quantitating selected acetates present in commercial cigarette tobacco. In this report, we demonstrate that the present method is capable of providing linear behavior over a wide range of concentrations, reasonable detection limits, and good reproducibility for fairly volatile compounds. Fast, reliable, unattended operation was possible by using an autosampler and the GC–MS system to obtain quantitative information when using appropriate internal standards. Differences observed for specific acetates, such as ethyl and *n*-propyl acetate, for various manufacturers would seem to preclude the notion that these chemicals are equally present in native tobacco, but instead reflect differences due to processing of the tobacco or in the manufacturing procedure. The high variability of concentration levels observed for different packs of the same brand may be due in part to the shelf life of that product. Therefore, the levels of acetates that we detected for individual brands should not be viewed as definitive, but rather should be seen as reflecting a lower estimate of the possible concentrations currently present in various commercial tobacco products.

Acknowledgments

The authors would like to acknowledge financial support for this study from both the National Center for Chronic Disease Prevention and Health Promotion and the Office of Smoking and Health.

References

1. J.M. McGinnis and W.H. Foege. Actual causes of death in the United States. *J. Am. Med. Assoc.* **270:** 2207–2212 (1993).
2. D. Hoffmann and I. Hoffmann. Tobacco smoke components. *Beit. zur Tabak.* **18:** 49–52 (1998).
3. D.L. Roberts. Natural tobacco flavor. *Recent Adv. Tob. Sci.* **14:** 49–81 (1988).
4. M.F. Dube and C.R. Green. Method of collection of smoke for analytical purposes. *Recent Adv. Tob. Sci.* **8:** 48–102 (1982).
5. First-quarter 1998 sales estimates for the cigarette industry. *The Maxwell Consumer Report.* Davenport & Company, LLC, Richmond, VA, May 18, 1998.
6. Z. Zhang, M.J. Yang, and J. Pawliszyn. Solid phase microextraction. *Anal. Chem.* **66:** 844A–853A (1994).
7. Z. Zhang and J. Pawliszyn. Headspace solid-phase microextraction. *Anal. Chem.* **65:** 1843–52 (1993).
8. K. Elke, E. Jermann, J. Begerow, and L. Dunemann. Determination of benzene, toluene, ethylbenzene and xylenes in indoor air at environmental levels using diffusive samplers in combination with headspace solid-phase microextraction and high-resolution gas chromatography–flame ionization detection. *J. Chromatogr.* **826:** 191–200 (1998).
9. R.B. Gaines, E.B. Ledford, and J.D. Stuart. Analysis of water samples for trace levels of oxygenate and aromatic compounds using headspace solid-phase microextraction and comprehensive two-dimensional gas chromatography. *J. Microcol. Sep.* **10:** 597–604 (1998).
10. M.N. Sarrion, F.J. Santos, and M.T. Galceran. Strategies for the analysis of chlorobenzenes in soils using solid-phase microextraction coupled with gas chromatography ion trap mass spectrometry. *J. Chromatogr.* **819:** 197–209 (1998).
11. K. Takekawa, M. Oya, A. Kido, and O. Suzuki. Analysis of

cyanide in blood by headspace solid-phase microextraction (SPME) and capillary gas chromatography. *Chromatographia* **47:** 209–214 (1998).
12. M. Mestres, O. Busto, and J. Gausch. Headspace solid-phase microextraction analysis of volatile sulphides and disulphides in wine aroma. *J. Chromatogr.* **808:** 211–18 (1998).
13. T.J. Clark and J.E. Bunch. Qualitative and quantitative analysis of flavor additives on tobacco products using SPME–GC–mass spectroscopy. *J. Agric. Food Chem.* **45:** 844–49 (1997).
14. T.J. Clark and J.E. Bunch. Quantitative determination of phenols in mainstream smoke with solid-phase microextraction–gas chromatography–selected ion monitoring mass spectrometry. *J. Chromatog. Sci.* **34:** 272–75 (1996).
15. S.S. Yang and I. Smetena. Determination of tobacco alkaloids using solid phase microextraction and GC–NPD. *Chromatographia* **47:** 443–48 (1998).
16. D.L. Ashley and J.D. Prah. Time dependence of blood concentrations during and after exposure to a mixture of volatile organic compounds. *Arch. Environ. Health* **52:** 26–33 (1997).
17. D.L. Ashley, M.A. Bonin, B. Hamar, and M.A. McGeehin. Removing the smoking confounder from blood volatile organic compounds measurements. *Environ. Res.* **71:** 39–45 (1995).
18. D.L. Ashley, M.A. Bonin, F.L. Cardinali, J.M. McCraw, J.S. Holler, L.L. Needham, and D.G. Patterson, Jr. Determining volatile organic compounds in human blood from a large sample population by using purge and trap gas chromatography/mass spectrtometry. *Anal. Chem.* **64:** 1021–29 (1992).
19. J.C. Leffingwell, H.J. Young, and E. Bernasek. *Tobacco Flavorings for Smoking Products.* R.J. Reynolds Tobacco Company, Winston-Salem, NC, 1972, pp 1–72.
20. P.H. Cramer, K.E. Boggess, and J.M. Hosenfeld. Volatile organic compounds in whole blood-determination by heated dynamic headspace purge and trap isotope dilution GC/MS. EPA-560/5-87-009, U.S. Government Printing Office, Washington DC, 1987.
21. B.N. Colby and M.W. McCaaman. A comparison of calculation procedures for isotope dilution determinations using gas chromatography mass spectrometry. *Biomed. Mass Spectrom.* **6:** 225–30 (1979).
22. J.K. Taylor. *Quality Assurance of Chemical Measurements.* Lewis Publishers, Chelsea, MI, 1987, pp 79–82.

Manuscript accepted February 18, 2000.