Improved Real-Time Puff-by-Puff GC–MS System for Whole Smoke Analysis

C.B. Huang and **David A. Self**
Philip Morris USA Research Center, 4201 Commerce Road, Richmond, VA 23234

Abstract

An automated puff-by-puff mainstream smoke (MSS) system is developed to monitor real-time whole smoke in mainstream cigarette smoke using gas chromatography (GC)–mass spectrometry (MS). The whole-smoke analysis is based on automated sample collection and injection into the GC–MS system. The important feature of this system is the real-time rapid analysis that is simple, sensitive, precise, flexible, and exhibits low carryover of volatile and semivolatile smoke constituents. The system is equipped with an automated sampling and switching valve and a smoking machine. The key improvements of the system, as compared with current and alternative methodologies, include minimizing variations caused by operator sampling techniques, the real-time analysis of MSS, the detection of flavorants in MSS from a single puff of cigarette smoke, the ability to analyze numerous smoke constituents from either whole smoke or the gas phase of a single puff, the ability to monitor a few selected smoke constituents in whole smoke using multiple puffs, and its good feasibility compared with solvent extraction and impinger trapping procedures for volatile organic compounds in MSS. System configuration and sampling methodologies are described. Sensitivity, flexibility, precision, feasibility, carryover, and applications of the system are discussed.

Introduction

Analytical chemists in the tobacco industry are continuously challenged to develop rapid and sensitive methods for testing of conventional and novel cigarette products. Improvements to established methods of investigation can hasten the completion of research projects and can also provide guidance for further investigations of prototype cigarettes under development. Research tools, such as puff-by-puff gas chromatography (GC) with the mass spectrometer (MS) (1,2) and Fourier transform IR (FTIR) (3,4) have been utilized for testing prototype cigarettes for their potential in reducing harmful constituents in cigarette smoke. The use of puff-by-puff GC–MS provides better sensitivity and selectivity for analyzing mainstream cigarette smoke (MSS), compared with the puff-by-puff FTIR method, which is limited to the analysis of gaseous compounds. Furthermore, the GC–MS system has better separation capability than the FTIR system for monitoring the complex matrix of cigarette smoke. More than 100 whole smoke constituents in a single puff from a cigarette analyzed by puff-by-puff GC–MS have been reported (2).

Reported here is an improved, real-time, puff-by-puff GC–MS system, which not only combines the capabilities of two previously described GC–MS procedures (1,2) for gas-phase and whole-smoke constituents, but also demonstrates the simplicity of using an automated valve system to minimize sampling variation, provides good precision and flexibility for a variety of samples, improves sensitivity with multiple puff injections from a single cigarette, and reduces carryover of constituents by using a helium/solvent back flush system. Volatile and semivolatile smoke constituents and flavorants were monitored by single-puff analysis using the fourth-puff screening method. The multiple-puff screening method, which can analyze eight puffs of smoke from a single cigarette, was used to monitor the concentration distribution of other smoke constituents and flavor-related compounds among puffs, and to measure the low concentrations of other semivolatile constituents, such as polyaromatic hydrocarbons (PAHs).

Experimental

Materials and samples

The test cigarettes used in this study were standard Kentucky reference cigarettes 2R4F (Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY). Cambridge pads (44 mm) were obtained from Whatman (Maidstone, U.K.). Grade-6 helium (99.9999% purity) (BOC Gases, Murray Hill, NJ) was used as the carrier gas and the back flush gas. Methanol (Optima, Fisher Scientific, Pittsburgh, PA) was used as the back-flush solvent.

Instrumentation

The GC–MS instrumentation consisted of a Shimadzu GC-2010 oven connected to a Shimadzu QP2010 mass selective detector (Shimadzu Scientific Instruments, Inc., Columbia, MD). The smoking machine, which was designed and developed by Philip Morris USA (PM USA, Richmond, VA), was a single-port machine

using a square-wave puff-volume profile. Sample introduction was performed using a Valco 6-port switching valve (Model DL6UWE, Valco Instruments Co., Inc., Houston, TX). Helium/solvent back-flush was performed using a Valco 3-port switching valve (Model ET3UWE). Cigarettes were lit using a Borgwaldt Technik electric lighter (Model R29, KC Automations, Richmond, VA). An Agilent DB-5 ms (60-m × 0.25-mm i.d., 1.0-µm film) column (No. 122-5563, Agilent Technologies, Inc., Palo Alto, CA) was used for this study.

Chromatography conditions

Single-puff analysis

For single-puff analysis (used in the fourth-puff screening method described later), the programmed temperature vaporization (PTV) injector was programmed at 120°C for 5 min, 260°C for 20 min, and 300°C for 11 min. The split ratio was 20:1 and the column flow rate was 1.0 mL/min. The oven was programmed for an initial temperature of 30°C for 8 min and ramped to 115°C at 5°C/min, held for 0 min, ramped to 290°C at 25°C/min, held for 12 min, ramped to 310°C at 10°C/min, and held for 4 min. The total run time was 75 min. The temperatures of the interface and ion source of the MS were 280°C and 240°C, respectively. Masses ranging from 20 to 350 amu were scanned from 4 to 75 min. A standard spectral autotune was accomplished with perfluorotributylamine (PFTBA) reagent.

Multiple-puff analysis

For multiple-puff analysis, the PTV injector was programmed at 150°C for 10 min and 280°C for 20 min. The split ratio was 200:1. The column flow rate was 2.0 mL/min, and the purge flow was 2.5 mL/min. The oven was programmed for an initial temperature of 150°C for 20 min, ramped to 180°C at 5°C/min, held for 2 min, ramped to 320°C at 25°C/min, and held for 15 min. The total run time was 48.6 min. The temperatures of the interface and ion source of the MS were 280°C and 240°C, respectively. Masses ranging from 29 to 300 amu were scanned from 4 to 48 min. A standard spectral autotune was accomplished with PFTBA reagent.

Heated automated sampling and injection system

The heated automated injection procedure for the method was established using a 6-port switching valve and a 3-port switching valve, equipped with a singleport smoking machine set at modified Federal Trade Commission smoking conditions (i.e., 35-mL puff volume, 2-s puff duration, and 60-s puff interval) using a square-wave puff profile. The 6-port switching valve was used to collect MSS from a sample cigarette. The MSS collected by 2 mL of the sample loop from the 35 mL of MSS was automatically delivered into the injector port of the GC–MS system, as schematically shown in Figures 1 and 2. The temperature of the 6-port switching valve was continuously maintained at 150°C to avoid any potential MSS condensation prior to the injection. Depending on the application, the split ratios of the GC injector were set at 20:1 or at 200:1 for better resolution. As depicted in Figure 3, a 3-port switching valve was used to introduce 15 mL of methanol with 850 mL/min of helium gas to flush MSS residues out of the 6-port switching and sampling system after analysis. The 3-port switching valve is referred to as the helium/solvent back-flush valve; it can also be used as an internal standard delivery device, as reported by Takanami (5).

Sampling and switching valve settings

Fourth-puff screening method

The switching valve was set at sampling mode at 3.5 min. At 4.03 min, it was set to injection mode and then back to sampling mode at 4.24 min. These settings enabled the sampling of a single puff of MSS on the fourth puff of a cigarette. At 4.43 min, the helium/solvent back-flush valve was turned on, having a helium flow of 850 mL/min; it was deactivated at 25 min.

Eight-puff screening method

The switching valve was set at sampling mode at 0.5, 1.24, 2.24,

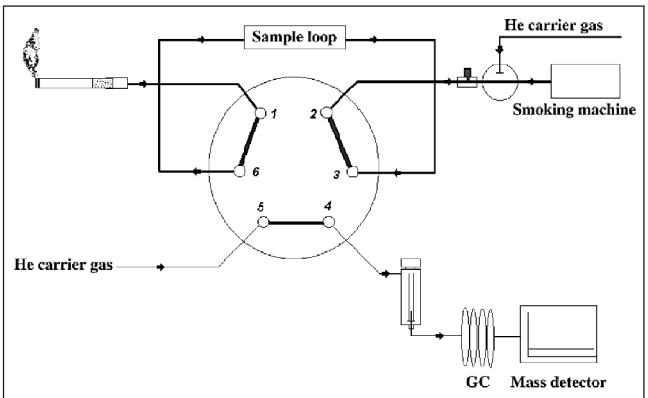

Figure 1. Valco switching and sampling valve at sampling mode.

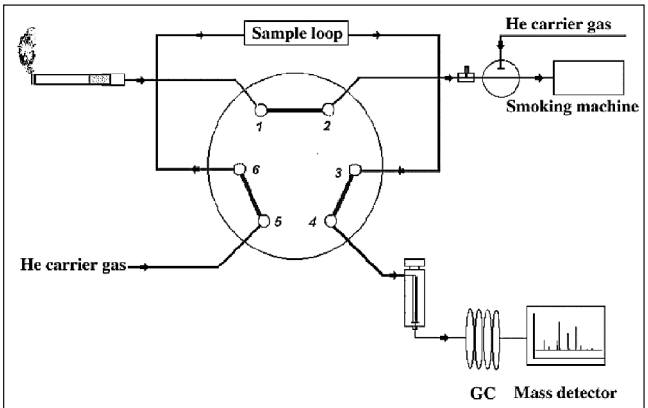

Figure 2. Valco switching and sampling valve at injection mode.

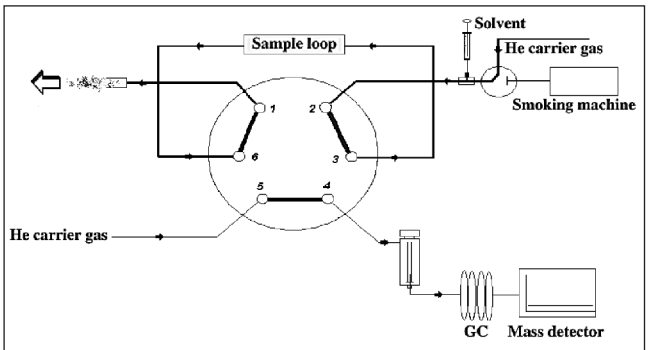

Figure 3. Valco switching and sampling valve at solvent back-flush mode.

3.34, 4.24, 5.24, 6.24, 7.24, and 8.24 min. The valve was set to injection mode at 1.03, 2.03, 3.03, 4.03, 5.03, 6.03, 7.03, and 8.03 min. These settings enabled the sampling of eight puffs of MSS from a single cigarette. At 8.43 min, the helium/solvent backflush valve was turned on, having a helium flow of 850 mL/min; it was deactivated at 25 min.

Smoke-puff profile

The port size of the rotor of the switching valve was a critical factor in the smoke puff volume and profile. The raw puff volume profiles from the smoking machine shown in Figures 4 and 5 illustrate that the puff volume reached approximately 95% of the designed value (35 mL) when using the sampling valve rotor with a port size of 1.7 mm at a duration time of 2 s, whereas the puff volume from the sampling valve rotor with a port size of 0.75 mm could only reach approximately 75% of 35 mL puff volume from the smoking machine. The distortion was believed to be associated with the pressure resistance from the smaller port size of the valve. The distortion of the puff volume may affect the smoke chemistry in MSS because of the burning temperature. Thus, the rotor with a port size of 1.7 mm was used in the system to avoid the distortion of the puff volume.

Sampling procedures

The sample cigarette was lit with the electric lighter, and, using a puff volume of 35 mL and a puff duration of 2 s at intervals of 60 s, whole smoke was collected using a single-port smoking machine that generated a square-wave profile. The sampling and switching valve, equipped with a 2-mL sample loop, was heated at 150°C. The transfer line connecting the sampling valve to the injection port of the GC was heated at 220°C to avoid smoke condensation and carryover of constituents. At exactly 1.8 s during a puff, the valve switched and injected an aliquot from the 2-mL sample loop of whole smoke into the GC injection port for analysis. After the injection of the selected puff, 15 mL of methanol was introduced to the sampling valve system with 850 mL/min of helium gas to flush out the condensed smoke constituents remaining in the sampling loop in order to minimize carryover to subsequent runs. The sampling valve system was purged with helium at 150°C for an additional 25 min to ensure that the valve system was dry prior to the next injection. (Figure 3).

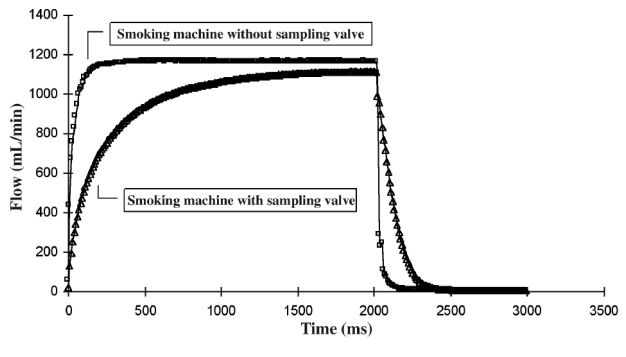

Figure 4. Raw puff volume profile for the 1.7-mm rotor.

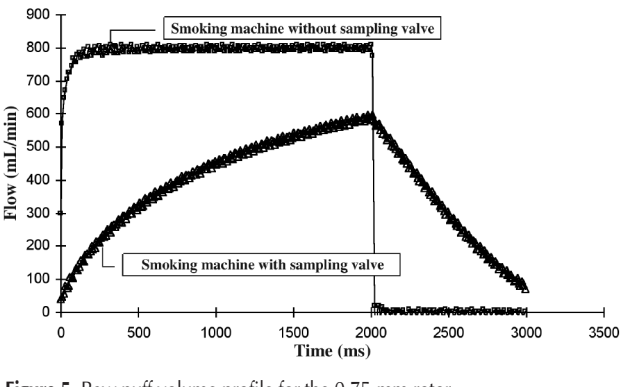

Figure 5. Raw puff volume profile for the 0.75-mm rotor.

Quantitation procedures

One-hundred and six whole smoke constituents have been identified and reported (2) using a manual injection puff-by-puff GC–MS method (2). Similar to the manual injection method, the automated puff-by-puff GC–MS can achieve the same goal for the analysis of MSS. Currently selected whole-smoke compounds were monitored for prototype cigarettes, which are experimental cigarettes currently under development for low constituent delivery. Analyte concentrations, measured in area counts, were reported as percentage reduction versus a control. Chemical identification was made by using the Wiley 7N and NIST98 libraries search results. These identifications should be considered tentative and confirmation should be made by actual reference materials. Kentucky reference 2R4F cigarette smoke, itself, served as a second standard.

Results and Discussion

Precision

The fourth puff of MSS from Kentucky reference 2R4F cigarette was used for the precision study. The GC–MS system and puff volume were calibrated prior to each analysis. Inter- and intraday variation for three selected major constituents of MSS are reported in Table I, which shows that the system demonstrated good inter- and intraday reproducibility from a single puff of a cigarette.

Sensitivity

As seen in Figures 6 and 7, the system has a great analytical sen-

Table I. Precision of the Real-Time Puff-by-Puff GC–MS System for the Selected Constituents, Nicotine, Neophytadiene, and Menthol

	CV (%)*		
	Day 1 (N = 4)†	Day 2 (N = 5)†	Day 3 (N = 5)†
Nicotine	7.6%	4.2%	6.7%
Neophytadiene	11.2%	9.3%	9.0%
Menthol	9.4%	10.8%	11.2%

* Relative standard deviation is expressed as percent coefficient of variation (CV).
† Number (N) of analytical runs per day.

sitivity for PAHs from the four-puff method. The system also shows a good sensitivity for volatile organic compounds (VOCs) and flavorants in MSS using a single puff from a cigarette. The GC profile of known flavors that were detected using the fourth-puff screening method with a selective ion mode (SIM) mass detector is shown in Figure 8.

Feasibility

The data obtained for VOCs in MSS of 2R4F cigarette samples from the puff-by-puff GC–MS system were compared with those from a cryogenic solvent trapping (STP) GC–MS procedure used in PM USA laboratories. Comparisons between the two methods are outlined in Table II. One of the major differences between the methods was the sample size. The ratio of the number of puffs used for the quantitation of the data between the STP and the puff-by-puff method was 29.4, suggesting that the variation from the puff-by-puff method would be 5.4 times greater than the STP, based on the ensemble averaging theory. However, the percentage of relative standard deviation for selected analytes by the puff-by-puff method was less than five-fold, compared with that of the STP (Figure 9). The data demonstrate that the precision of the puff-by-puff method was comparable with that of STP. Therefore, the puff-by-puff method is a feasible method to use for the rapid screening measurement of VOCs in MSS.

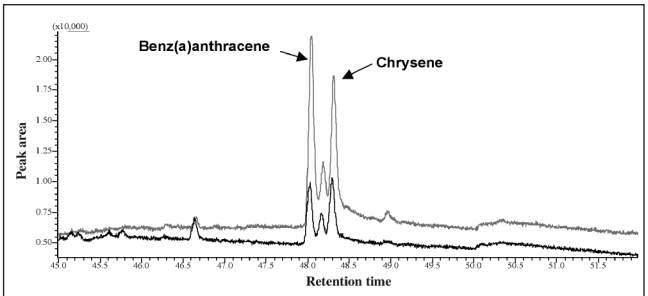

Figure 6. GC profile of benz(a)anthracene in mainstream smoke.

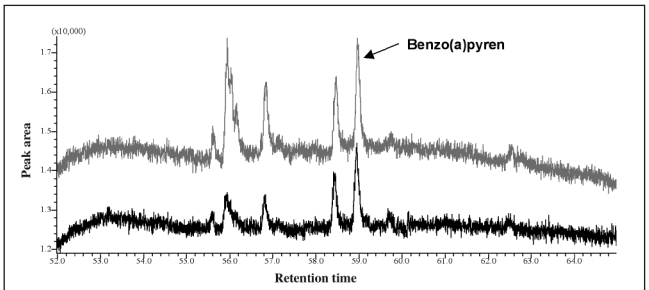

Figure 7. GC profile of benzo(a)pyrene in mainstream smoke.

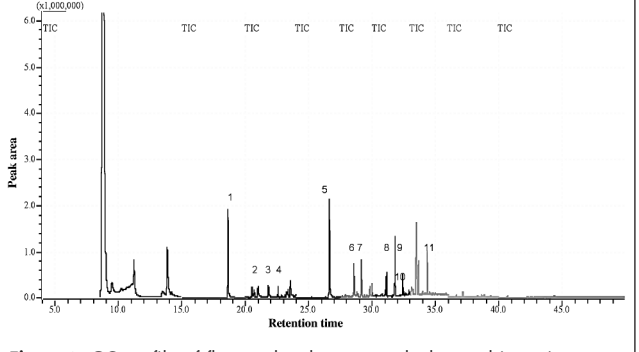

Figure 8. GC profile of flavor-related compounds detected in mainstream smoke. (1 propylene glycol; 2 2,5-dimethyl pyrazine; 3 valeic acid; 4 limonene; 5 benzyl alcohol; 6 phenylethyl alcohol; 7 α-terpineol; 8 carvone; 9 piperitonr; 10 ethyl carprate; and 11 citronelly isobutyrate)

Flexibility

The improved puff-by-puff GC–MS system combined two GC–MS procedures previously developed by scientists at PM USA for gas-phase and whole-smoke constituent analyses. With or without a Cambridge pad at the smoking port, smoke constituents of gas phase or whole smoke was determined by the system. The improved system also demonstrated its ability to monitor constituents from one puff to up to eight puffs using one cigarette. This multiple-puff capability provided great analytical benefits for monitoring flavorant distribution between puffs, such as menthol, for flavor-system development in the production of cigarettes (Figure 10).

Table II. Sampling Parameters for the Feasibility Study of the Real-Time Puff-by-Puff GC–MS System

Analytical method	Cigarettes used (*N*)	Puffs/cigarette mean	Replicate	Total puffs analyzed
Solvent trapping (STP)	10	9.8	3	294
Puff-by-puff GC–MS	1	1.0	10	10

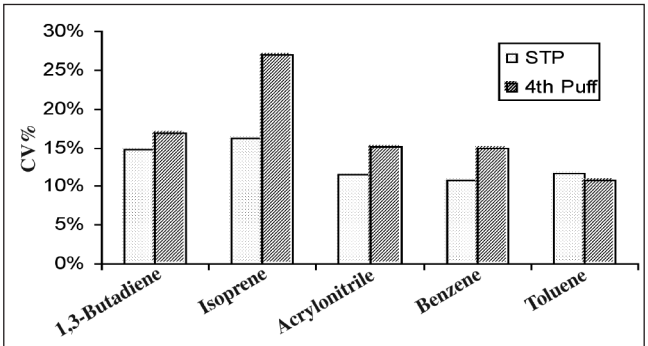

Figure 9. Percentage of relative standard deviation (as % coefficient of variation) of the puff-by-puff method compared with the solvent trapping method for the analysis of volatile organic compounds.

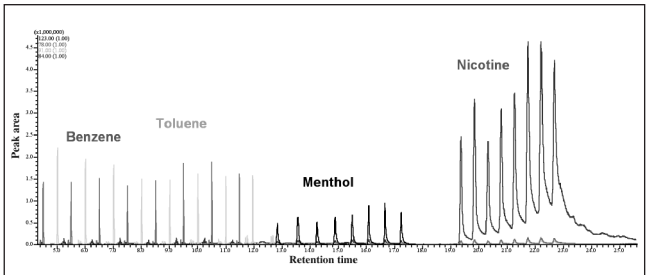

Figure 10. Eight puffs of benzene, toluene, menthol and nicotine in mainstream smoke from a single cigarette.

Carryover

Carryover is always a big concern when chromatography systems, such as gas or liquid chromatography, are used in the analysis of MSS. The particulate phase or semivolatile constituents in MSS can be trapped in the sampling loop, sampling valve, or transfer line of the GC, even at an elevated temperature. This is the main reason for a solvent back flush, which will reduce or eliminate potential carryover problems in subsequent analytical runs. Analysis of a blank sample injected into the system following the helium/solvent back flush showed that the solvent back flush can eliminate the carryover of volatile and semivolatile constituents of MSS, such as 1,3-butadiene, acrolein, toluene, styrene, menthol, triacetin, and nicotine. However, high-molecular-weight constituents, such as PAHs, persisted even after the helium/solvent back flush. Fortunately, this problem was minimized by running two blank samples after the MSS analysis and the subsequent back flush. The analysis of a third blank sample resulted in no significant detectable amounts of PAHs.

However, this investigation also indicated that a certain amount of PAHs accumulated on the PTV injector or chromatography column head after the system was idle during the overnight period. This suggested that the helium/solvent back flush was unable to completely remove the PAHs from the sampling system (the switching valve and transfer line). Therefore, we concluded that a small amount of PAHs will slowly evaporate and migrate to the injector after a long period of time when the valve temperature is 150°C to 160°C. Back flushing the sampling valve and baking the column and injection port are strongly recommended when the system is idle for a long period of time.

Conclusion

This study shows that the real-time puff-by-puff GC–MS system is a flexible and precise method to monitor smoke constituents in MSS from a cigarette. The automated puff-by-puff method is sensitive and is capable of monitoring VOCs, PAHs, menthol, and flavor-related compounds in MSS using a single puff or multiple puffs from a single cigarette. The carryover of volatile compounds, such as the VOCs in MSS, was eliminated using the helium/solvent back-flush system. However, carryover of the high-molecular-weight semivolatile smoke constituents, such as the PAHs, was observed. Baking the column or running two blank analyses is necessary for analysis of PAHs in MSS. Overall, the results of this investigation show that the automated puff-by-puff GC–MS system is a rapid and feasible research tool when only a limited number of samples are available.

Acknowledgments

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