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Simultaneous Analysis of 22 Volatile Organic Compounds in Cigarette Smoke Using Gas Sampling Bags for High-Throughput Solid-Phase Microextraction

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Supporting Information

ABSTRACT: Quantifying volatile organic compounds (VOCs) in cigarette smoke is necessary to establish smoke-related exposure estimates and evaluate emerging products and potential reduced-exposure products. In response to this need, we developed an automated, multi-VOC quantification method for machine-generated, mainstream cigarette smoke using solid-phase microextraction gas chromatography−mass spectrometry (SPME-GC−MS). This method was developed to simultaneously quantify a broad range of smoke VOCs (i.e., carbonyls and volatiles, which historically have been measured by separate assays) for large exposure assessment studies. Our approach collects and maintains vapor-phase smoke in a gas sampling bag, where it is homogenized with isotopically labeled analogue internal standards and sampled using gas-phase SPME. High throughput is achieved by SPME automation using a CTC Analytics platform and custom bag tray. This method has successfully quantified 22 structurally diverse VOCs (e.g., benzene and associated monoaromatics, aldehydes and ketones, furans, acrylonitrile, 1,3-butadiene, vinyl chloride, and nitromethane) in the microgram range in mainstream smoke from 1R5F and 3R4F research cigarettes smoked under ISO (Cambridge Filter or FTC) and Intense (Health Canada or Canadian Intense) conditions. Our results are comparable to previous studies with few exceptions. Method accuracy was evaluated with third-party reference samples (≤15% error). Short-term diffusion losses from the gas sampling bag were minimal, with a 10% decrease in absolute response after 24 h. For most analytes, research cigarette inter- and intrarun precisions were ≤20% relative standard deviation (RSD). This method provides an accurate and robust means to quantify VOCs in cigarette smoke spanning a range of yields that is sufficient to characterize smoke exposure estimates.

Cigarette smoke is the primary source of exposure to several VOC carcinogens and toxicants in the U.S. general population.[1] Specifically, four VOCs, benzene, 1,3-butadiene, acrylonitrile, and acetaldehyde, have been ranked in the top five cancer risk constituents of tobacco smoke[2] and many others have been classified as developmental toxicants.[3] Although quantifying VOCs in mainstream cigarette smoke presents many analytical challenges, this analysis is needed to provide smoker exposure estimates and evaluate new products and potential reduced-exposure products.

Machine smoking does not represent individual smoker behavior, but it is a reproducible means to control smoking conditions for product comparison or exposure estimation. In the smoking machine, mainstream smoke is fractionated into a particulate phase (i.e., particulate matter) that collects onto a fiber-glass filter pad and a vapor phase that passes through the filter pad. Although most VOCs exist in the vapor phase, less volatile and more polar VOCs can partition into the particulate matter or condense onto the filter pad as part of the particulate phase. Accurate VOC quantification in these phases requires proper sample handling and internal standardization to overcome the analytical challenges inherent to working with high concentration volatiles in a complex matrix. These challenges include loss processes that occur during sample handling and collection (e.g., volatilization, adsorption, reactivity) and residue carryover from adsorption and condensation within the smoking apparatus.

VOC analysis of the vapor phase can be performed on the intact vapor or vapor condensed in a trap. The most common trapping techniques use solvent-based impinger (e.g., methanol)[4,5] or solid sorbent (e.g., Tenax)[6,7] traps. These traps are most effective for trace analysis because they are susceptible to breakthrough of high concentration compounds, especially nonpolar volatiles. Breakthrough losses can be reduced by cryogenic cooling; however, volatile or highly concentrated compounds may still exceed the cooled trap's loading capacity and require a series of traps for complete collection.[4,5]

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Cryogenic cooling also reduces losses from reactivity and decomposition within the trap, but internal standardization is necessary for quantitative analysis. For many sorbent and impinger trapping methods, internal standards (ISTDs) are added to the trap after collection and are not able to compensate for losses that occur as the vapor phase condenses. Charles et al. published one of the most encompassing methods, reporting 36 VOCs using thermal sorption/desorption traps and two fluorinated benzenes as surrogate ISTDs.[7] Charles et al. were able to measure many semivolatile VOCs (e.g., naphthalene), but their traps could not capture small (≤4 carbon) carbonyl compounds. Small carbonyls are important to measure because they are the most abundant VOCs in cigarette smoke, though they are difficult to quantify due to their high volatility and reactivity. For more reactive compounds, such as carbonyls, impingers[8,9] or sorbent traps[10] are used in conjunction with derivitization techniques (e.g., 2,4-dinitrophenylhydrazine). Impingers require significant solvent, introducing waste management costs.

Direct analysis of the intact vapor phase minimizes sample handling and maintains the sample in a gaseous state amenable to gas chromatography−mass spectrometry (GC−MS). Direct analysis methods have used a short gas sampling loop connecting the smoke generating system directly in-line with the analytical instrument to perform puff-resolved analysis seconds after smoke generation.[11−15] The advantages of this approach are decreased container surface area interaction and decreased sample aging. However, in-line methods require extensive modifications to create the sampling loop[16] and chromatography-based methods are limited to single puff analysis due to throughput restrictions.[12] As an alternative to in-line analysis, vapor-phase smoke from a whole cigarette can be collected into an inert gas sampling bag/canister and a small volume of the sample can be analyzed.[17] The addition of isotopically labeled analogue ISTDs at the time of sample collection can capture handling and aging losses.

Intact vapor-phase sampling can also be performed using solid phase microextraction (SPME), a solvent-free technique that selectively extracts compounds from a complex matrix onto a sorbent fiber.[18] Gas-phase SPME sampling has been successful for previous smoke analyses.[19−21] Isotopically labeled analogue ISTDs enable accurate quantification when SPME efficiency is adjusted (e.g., sorbent material, collection time) to eliminate the need for sample dilution. Furthermore, SPME of the intact vapor phase eliminates potential breakthrough issues associated with sorbent and impinger trapping.

We developed an automated, high-throughput, and accurate method to directly analyze machine-generated mainstream cigarette smoke using SPME-GC−MS. Unlike previous SPME methods that involved manually transferring the vapor phase into a headspace vial before extraction,[19,21] our approach is less labor intensive and reduces sample handling. To achieve this, smoke particulate is collected onto a filter pad and the vapor phase is collected into a Tedlar (polyvinylfluoride) gas sampling bag. The gas sampling bags are chemically inert and have low permeability to VOCs. Isotopically labeled analogue internal standards (ISTDs) are important for accurate quantification because the broad range of VOCs measured have individual differences in reactivity, surface interaction with the gas sampling bag, SPME efficiency, and MS ionization efficiency. The 22 hazardous VOCs targeted by this method are toxicologically relevant and 16 have been identified by the FDA as harmful or potentially harmful constituents in tobacco products and tobacco smoke. The current approach quantifies both carbonyls and volatiles, which historically have been measured by separate assays, thus increasing the number and diversity of toxic VOCs quantified relative to previous methods. This method involves little solvent and minimal sample preparation, which makes it well-suited for high-throughput analyses, such as multiregimen and multireplicate studies.

EXPERIMENTAL SECTION

Standards. Calibration neat materials were purchased from Sigma-Aldrich (St. Louis, MO) as liquids in flame-sealed ampules with ≥97% chemical purity, except 2-nitropropane (95% chemical purity). Calibration intermediates at nine concentration levels were prepared by dissolving gravimetrically determined amounts of each compound into purge-and-trap (P&T) grade methanol (o2si smart solutions, Charleston, SC). The more reactive analytes, 1,3-butadiene, acetaldehyde, acrylonitrile, vinyl acetate, and vinyl chloride, were prepared in a separate solution. Aliquots of the calibration intermediates were stored in flame-sealed ampules at −70 °C for long-term storage or at −20 °C for a maximum of 2 weeks. Appropriate personal protective equipment was used when handling neat materials and calibration solutions.

Intermediate solution ampules were brought to room temperature and sonicated to ensure complete mixing before use. Vapor-phase calibrators were prepared by transferring 10 μL of both reactive and nonreactive intermediate calibration solutions by gastight syringe into a gas sampling bag containing 350 mL of air and 40 μL of ISTD. The vapor-phase calibrator bags were heated in an oven at 50 ± 5 °C for 60 ± 5 min to ensure rapid and complete homogenization. The particulate-phase intermediate calibration solutions were made by diluting both intermediate ISTD and calibration solutions together into P&T grade methanol to achieve a 24-fold dilution for each solution. From the resulting solution, 20 μL was spiked onto a new pad in a 20 mL headspace vial, which was crimp sealed immediately.

The calibrators were formulated to encompass literature values for VOCs in mainstream cigarette smoke. In the vapor phase, calibration ranged from 0.050 to 158 μg/bag for all analytes except vinyl chloride (0.005−50.0 μg/bag) and furan, methylvinylketone, 2,3-butanedione, methylethylketone, benzene, and ethylbenzene (0.050−500 μg/bag). To accommodate Intense samples, calibrator concentrations were doubled by spiking twice the amount of intermediate solution. Additionally, three supplemental calibration levels were included for acetaldehyde (12 600 μg/bag), toluene (1000 μg/bag), and 1,3-butadiene (200 μg/bag).

Calibration accuracy was confirmed by third-party reference samples obtained from a company with International Organization for Standardization (ISO, Geneva, Switzerland) Guide 34 certification (Sigma-Aldrich). If reference sample results erred >15%, a validation mix containing the compound in question and a control was formulated by another vendor (Absolute Standards, Inc., Hamden, CT) for comparison and correction.

Internal Standards. Isotopically labeled analogues were purchased from Sigma-Aldrich, C/D/N Isotopes, Inc. (Pointe-Claire, Quebec, Canada), and Cambridge Isotope Laboratories, Inc. (Tewksbury, MA) as neat liquids in flame-sealed ampules with ≥98% isotopic purity and ≥97% chemical purity, except [$^2H_6$]crotonaldehyde (95% chemical purity). Some compounds required custom synthesis. As with the calibration solutions,

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separate reactive and nonreactive solutions were prepared gravimetrically (o2si smart solutions). Aliquots of the ISTD were stored in flame-sealed ampules at −70 °C. Each vapor-phase bag was spiked with 40 μL of ISTD that was prepared as a 1:1 reactive to nonreactive ISTD mix. For the particulate phase, 20 μL of ISTD, diluted 24-fold, was added to each pad sample.

Cigarettes. Cigarettes were stored at room temperature for up to 3 months and at, or below, −16 °C for longer term storage. Prior to sampling, cigarettes were conditioned with ISO 3402:1999 recommended conditions of 22 °C and 60% relative humidity for at least 48 h, but less than 10 days, with the pack unsealed.

Quality Control Materials. Research cigarettes 3R4F and 1R5F (University of Kentucky, Lexington, KY) were used as quality control (QC) samples and were included in each smoking machine run. These QC cigarettes were smoked with 3 unlit clearing puffs collected after smoking was completed. Material blanks for the vapor phase were gas sampling bags filled with approximately 350 mL of laboratory air, equivalent to 10 × 35 mL puffs, and spiked with ISTD. Material blanks for the particulate phase were new filter pads inserted into a clean headspace vial and spiked with ISTD. Matrix blanks for both phases were collected as normal QC samples on the smoking machine, with empty, but filtered, cigarette holders. Matrix blanks accounted for carryover in the smoking machine and sidestream smoke contamination from cigarettes on neighboring ports. Cigarette and QC samples were accepted based on modified Westgard rules.[22] Nitrobenzene and 2-nitropropane deliveries were below the lowest calibrator in both research cigarettes.

Smoking Conditions. Cigarettes were smoked on an automated, linear 16-port ASM516 smoking machine (Cerulean, Milton Keynes, U.K.) with collection bags attached directly to the exhaust ports of the puffing engines. Cigarettes were smoked following the ISO 3308:2000 regimen (35 mL puffs with 2 s durations every 60 s, filter tip ventilation unchanged) and Intense regimen (55 mL puffs with 2 s durations every 30 s, filter tip ventilation blocked). For the Intense regimen, filter tip ventilation holes were blocked by wrapping two layers of cellophane tape around the filter. Three clearing puffs were collected for each sample after the last cigarette completed smoking. Cigarette samples and one matrix blank were collected with two pairs of 3R4F and 1R5F during each run.

Vapor Phase. The vapor phase was collected into airtight, inert polyvinylfluoride 1 L gas sampling bags (New Star Environmental, Roswell, GA) joined to the smoking machine using a short piece of PVC tubing and quick-disconnect coupling. These bags were fitted with butyl rubber O-rings. A random sampling from each lot of bags was analyzed to evaluate material residue levels, which were below the limit of detection for all analytes. Every bag was inspected for adequate compression of the O-rings. After inspection, each gas sampling bag was spiked with ISTD immediately (≤5 min) before sample collection. After collection, the vapor-phase unknown and QC samples were heated in an oven at 50 ± 5 °C for 60 ± 5 min to facilitate homogenization. The gas sampling bags were mounted on a custom CTC Analytics (Zwingen, Switzerland) combi-PAL bag tray (Leap Technologies, Carborro, NC) with three rows of five bags held in a single plane. Each bag is held in place by a clip that fastens around the injection port at the base of the septum fitting. A CTC rail equipped with a 75 μm carboxen-PDMS SPME fiber (Supelco, Bellefonte, PA) was used to extract VOCs from the gas sampling bag through the bag injection port for 1 min at room temperature.

Particulate Phase. Particulate-phase samples were collected onto preconditioned, 44 mm Cambridge type fiberglass pads (Performance Systematix Inc., Grand Rapids, MI). After smoke collection, the Cambridge filter pads were removed from the cigarette holders, placed into 20 mL headspace vials, spiked with ISTD, and sealed with PTFE-lined silicone septa crimp caps (Lab Depot, Dawsonville, GA). Headspace vials were stored on CTC combi-PAL trays at room temperature and sampled with a 75 μm carboxen-PDMS SPME fiber (Supelco) for 4 min at 40 °C without agitation.

Analytical Instrumentation. VOCs were quantified using two 6890 GC/5973 MSD instruments (Agilent Technologies, Palo Alto, CA) with the same GC parameters; one was configured with a Purged Ultimate Union (Agilent Technologies). For both systems, the GC inlet was 250 °C with a 50 psi pressure-pulsed splitless injection. The column was a 40 m DB-VRX capillary column with 0.18 mm I.D. and 1.0 μm film thickness (J&W Scientific, Folsom, CA). The carrier gas was research grade helium (Airgas, Inc., Radnor, PA) and the helium flow was controlled via pressure ramping. The oven was held at 0 °C for 1.5 min, then ramped to 140 °C (7 °C/min) and to 220 °C (40 °C/min), which was maintained for 1.5 min. The column pressure was held at 38 psi for 1.5 min, then ramped to 50 psi (0.6 psi/min) and to 66.63 psi (5.32 psi/min), which was held for 2.5 min. A purge flow of 100 mL/min was activated after 1.5 min. For the GC with the purged union, a pressure-controlled tee was installed at the end of the analytical column and 0.6 m of the 0.15 mm I.D. deactivated capillary column served as a restrictor and transfer line. A 5 min postrun cycle with an oven ramp to 220 °C and a 50 psi backflush was intended to clear the column of highly retained compounds; however, both instruments performed similarly. A liquid N_2 cooling trap (SIS, Ringoes, NJ) was installed at the head of the GC column to cryofocus at −100 °C for 1 min at the beginning of each analytical run before rapidly heating to 220 °C.

Mass spectrometry was performed using electron ionization and selective ion monitoring (SIM) with the source heated to 230 °C and the quadrupole to 150 °C. SIM masses (m/z) were selected for abundance and uniqueness to minimize spectral overlap with coeluting interferents. Two mass spectral ions, a primary quantification ion (1°) and secondary confirmation ion (2°), were monitored for each analyte to ensure correct identification. A third ion was monitored for each analogue ISTD and identification was confirmed by retention time gap with the native ions, visual inspection of the peak symmetry, and evaluation of sample-to-sample absolute response. The ions used for this study are listed in Table 1, but other ions may be suitable. A 30 ms dwell time was acceptable for most ions. Individual analyte concentrations were derived from the ratio of the native analyte response to the ISTD response using peak area for all analytes except furan, nitromethane, 2,3-butanedione, crotonaldehyde, and 3-pentanone, which used peak height to minimize interferences from chromatographically and spectrally unresolved compounds.

RESULTS AND DISCUSSION

Automation and General Approach. This automated method was developed to accurately quantify a broad range of hazardous VOCs in mainstream cigarette smoke for large

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Table 1. Analytical Parameters for Quantification[a]

analyte	CAS number	ISTD	1°, 2°, ISTD (m/z)
vinyl chloride	75-01-4	$^{2}H_3$	62, 64, 65
acetaldehyde	75-07-0	$^{2}H_4$	44, 43, 48
1,3-butadiene	106-99-0	$^{2}H_6$	54, 39, 60
furan	110-00-9	$^{2}H_4$	68, 39, 72
acrylonitrile	107-13-1	$^{2}H_3$	53, 52, 56
nitromethane	75-52-5	^{13}C	46, 61, 62
vinyl acetate	108-05-4	$^{13}C_2$	86, 43, 88
methylvinylketone (3-buten-2-one)	78-94-4	$^{2}H_8$[c]	55, 70, 58
butanal (butyraldehyde)	123-72-8	$^{2}H_8$	72, 57, 80
2,3-butanedione	431-03-8	$^{2}H_6$	86, 87, 92
methylethylketone (2-butanone)	78-93-3	$^{2}H_8$	72, 43, 80
crotonaldehyde (2-butenal)	4170-30-3	$^{13}C_4$	70, 41, 74
benzene	71-43-2	$^{13}C_6$	78, 51, 84
2-pentanone	107-87-9	$^{2}H_5$	86, 43, 91
3-pentanone	96-22-0	$^{2}H_{10}$	86, 87, 62
2-nitropropane[b]	79-46-9	$^{2}H_6$	43, 41, 45
2,5-dimethylfuran	625-86-5	$^{13}C_2$	96, 95, 98
toluene	108-88-3	$^{13}C_7$	91, 92, 98
ethylbenzene	100-41-4	$^{2}H_{10}$	106, 91, 98
m/p-xylene	108-38-3	$^{13}C_8$	106, 91, 98
styrene	100-42-5	$^{13}C_6$	104, 78, 110
o-oylene	95-47-6	$^{2}H_6$	106, 92, 112
3-ethyltoluene	620-14-4	$^{2}H_5$	105, 120, 107
nitrobenzene[b]	98-95-3	$^{13}C_6$	77, 123, 129

[a]Analytes are listed in elution order. [b]Not detected in research cigarettes. [c]Nonanalogue.

exposure assessment studies. As such, this method achieves high throughput, minimizes sample handling, and increases the number and diversity of toxic VOCs quantified. The current approach collects and maintains vapor-phase smoke in a gaseous state in a gas sampling bag, where it is homogenized with ISTDs and sampled using gas-phase SPME. Automated SPME sampling is accomplished on a CTC platform with a custom bag tray. The gas sampling bag injection port is physically compatible with the CTC SPME sampling process and can be joined to the smoking machine. Connecting the gas sampling bag to the smoking machine puff engine exhaust valve minimizes the sample path length from the cigarette to the gas sampling bag to less than 20 cm. This short sample path length reduces smoke condensation and adsorption within the smoking machine.

In this study, VOC deliveries (μg/cigarette) were quantified for both the vapor and particulate phases. Although collection of the vapor phase is relatively hermetic, particulate-phase samples require manual transfer of the pad from the cigarette holder to a SPME headspace vial. This manual transfer step increases the risk of volatilization loss. To eliminate this loss, clearing puffs are added to the end of each smoking run to draw residual VOCs from the pad into the gas sampling bag. Experiments with 1R5F and 3R4F research cigarettes smoked under the Intense regimen show that 3 clearing puffs are sufficient to lower the particulate-phase contribution to the total (vapor + particulate) VOC delivery below 5% for most analytes. The only exception was 3-ethyltoluene, which had 12% of total delivery remaining in the particulate phase due to its low volatility. Under Intense conditions, 3 clearing puffs (plus lit puffs) approached the capacity limitations of the 1 L gas sampling bag; however, larger volume gas sampling bags may be used if more clearing puffs are desired. As others have noted, we believe that the empirically defined vapor and particulate phases are arbitrary categorizations that may not represent two distinct exposures that occur during cigarette smoking. Therefore, although the filter pad is useful for smoking machine operation, quantification in both phases is unnecessary for the VOCs analyzed here if at least 3 clearing puffs are used. Particulate-phase results are not reported here because of the low contribution of the particulate phase to total delivery using this clearing approach.

Analyte-Specific Biases. Analyte-specific biases from adsorption, diffusion, volatilization and reactivity losses, competition effects and capacity limitations at the SPME fiber, and efficiencies of GC−MS processes (e.g., ionization efficiency) have been minimized through the use of appropriate sample handing and isotopically labeled analogue ISTDs. Experimentally, quantification was more precise when using response ratios with labeled analogue ISTDs compared to the absolute responses. Averaged across the analytes, the response ratio concentrations had 10.6% RSD and the absolute responses had 18.9% RSD for 14 replicate 3R4F cigarettes analyzed within the same smoking run under ISO conditions.

To ensure rapid and complete homogenization of the ISTD, especially for more polar and less volatile compounds such as 3-ethyltoluene and nitrobenzene, the gas sampling bags were heated immediately after sample collection. Although heating increases the permeability of the gas sampling bag material (including sampling port O-rings) and may increase losses through the punctured septa, rapid sample/ISTD homogenization ensures that the ISTD will compensate for all loss mechanisms that occur thereafter.

To minimize analyte-specific absorption by the gas sampling bag inlet O-rings, low permeability O-rings made with butyl rubber, rather than more permeable and more common silicone, were evaluated. We compared long-term change in absolute response for ethylbenzene, 2,5-dimethylfuran, and nitromethane using bags fitted with butyl and silicone O-rings for a 3R4F research cigarette vapor-phase sample analyzed over a 5 day (120 h) period (Figure 1). For this comparison, ISTD was not used, thus the bag septum was unpierced until the first analysis by the SPME needle. Comparison of absolute response

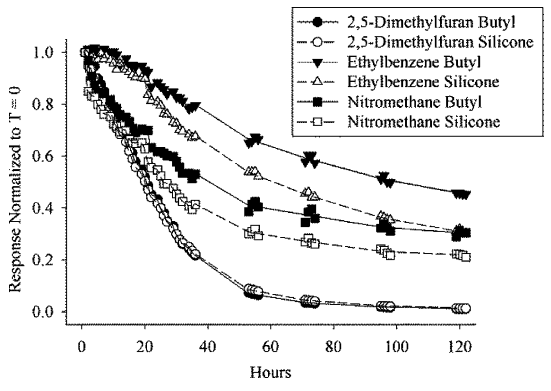

Figure 1. Change in absolute response for ethylbenzene, 2,5-dimethylfuran, and nitromethane using bags fitted with butyl O-rings, shown as filled symbols with a solid line, and silicone O-rings, shown as open symbols with a dashed line, for a 3R4F research cigarette vapor phase sample analyzed over a 5 day period.

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Table 2. ISO and Intense Regimen Results (μg/Cigarette) and Precision (%RSD) for 1R5F and 3R4F Research Cigarettes[a]

analyte	ISO 3R4F (μg/cig) mean ± SD (%RSD)	ISO 1R5F (μg/cig) mean ± SD (%RSD)	Intense 3R4F (μg/cig) mean ± SD (%RSD)	Intense 1R5F (μg/cig) mean ± SD (%RSD)
vinyl chloride	0.030 ± 0.005 (16%)	0.008 ± 0.002 (22%)	0.08 ± 0.009 (11%)	0.058 ± 0.009 (16%)
acetaldehyde	620 ± 127 (21%)	198 ± 51.4 (26%)	1740 ± 212 (12%)	1300 ± 251 (19%)
1,3-butadiene	36.7 ± 6.72 (18%)	12.1 ± 2.46 (20%)	92.4 ± 12.6 (14%)	84.2 ± 16.9 (20%)
furan	23.9 ± 3.71 (16%)	6.76 ± 0.943 (14%)	57.3 ± 4.66 (8.1%)	40.9 ± 4.36 (11%)
acrylonitrile	7.70 ± 1.22 (16%)	1.93 ± 0.388 (20%)	28.0 ± 2.46 (8.8%)	27.5 ± 3.33 (12%)
nitromethane	2.30 ± 0.591 (26%)	0.919 ± 0.349 (38%)	6.52 ± 0.783 (12%)	5.94 ± 0.822 (14%)
vinyl acetate	0.321 ± 0.046 (14%)	0.103 ± 0.018 (17%)	0.857 ± 0.073 (8.5%)	0.67 ± 0.06 (8.9%)
methylvinylketone	34.5 ± 7.37 (21%)	8.69 ± 2.09 (24%)	120 ± 32.7 (27%)	93.8 ± 27.5 (29%)
butanal	5.81 ± 0.646 (11%)	1.69 ± 0.281 (17%)	18.3 ± 2.28 (12%)	15.5 ± 2.55 (16%)
2,3-butanedione	64.1 ± 15.4 (24%)	18.5 ± 5.89 (32%)	248 ± 40.4 (16%)	187 ± 29.3 (16%)
methylethylketone	55.9 ± 6.56 (12%)	15.2 ± 2.35 (15%)	170 ± 16.1 (9.5%)	130 ± 18.0 (14%)
crotonaldehyde	9.51 ± 1.34 (14%)	1.67 ± 0.298 (18%)	41.5 ± 4.17 (10%)	30.9 ± 3.86 (13%)
benzene	36.3 ± 5.75 (16%)	11.3 ± 2.12 (19%)	97.7 ± 7.91 (8.1%)	75.0 ± 6.56 (8.7%)
2-pentanone	7.48 ± 0.937 (13%)	1.80 ± 0.198 (11%)	25.9 ± 2.22 (8.6%)	17.6 ± 1.89 (11%)
3-pentanone	3.02 ± 0.408 (13%)	0.620 ± 0.113 (18%)	10.8 ± 0.974 (9.0%)	7.24 ± 0.784 (11%)
2,5-dimethylfuran	14.3 ± 2.59 (18%)	2.61 ± 0.489 (19%)	39.1 ± 4.60 (12%)	16.4 ± 1.80 (11%)
toluene	41.1 ± 7.89 (19%)	9.22 ± 1.92 (21%)	139 ± 14.0 (10%)	101 ± 10.3 (10%)
ethylbenzene	3.34 ± 0.743 (22%)	0.712 ± 0.168 (24%)	16.4 ± 1.55 (9.4%)	11.8 ± 1.6 (13%)
m/*p*-xylene	5.86 ± 1.48 (25%)	1.31 ± 0.338 (26%)	27.5 ± 2.3 (8.4%)	17.4 ± 1.94 (11%)
styrene	1.94 ± 0.419 (22%)	0.444 ± 0.107 (24%)	14.5 ± 1.47 (10%)	11.1 ± 1.56 (14%)
o-xylene	1.08 ± 0.241 (22%)	0.242 ± 0.060 (25%)	5.81 ± 0.697 (12%)	3.63 ± 0.524 (14%)
3-ethyltoluene	0.438 ± 0.102 (23%)	0.102 ± 0.029 (28%)	3.04 ± 0.341 (11%)	1.92 ± 0.297 (15%)

[a]Analytes are listed in elution order.

over time without internal standardization does not compensate for analyte-specific competition or limited capacity at the fiber that might positively bias absolute VOC levels as competition decreases. Thus, this experiment only approximates relative analyte concentrations over time under the condition of repeated puncturing.

Experimentally, we observed a decrease in response over time, indicative of a single loss mechanism (Figure 1) presumed to be diffusion loss through the sample bag (i.e., punctured septum, O-rings, and Tedlar). Within the first 12 h for both bags, most analyte responses decrease no more than 10%, with an average loss of 2% across the analyte list; however, acrylonitrile, styrene, 2,5-dimethylfuran, and nitromethane responses decreased by approximately 25%. After 24 h, the average decrease in absolute response was 10% across the analyte list, but greater losses were observed for 2,5-dimethylfuran (57%), styrene (40%), and nitromethane (38%). All monoaromatics responded in the same manner as ethylbenzene with slower loss from the bags fitted with butyl rubber rather than silicone O-rings. For other compounds, differences between butyl and silicone O-rings within 24 h were small; however, after 2 days, relative VOC concentrations were significantly higher in bags with the butyl O-rings for vinyl acetate, nitromethane, styrene, 2-butenal, 3-ethyltoluene, and *o*-xylene. By day 3, diffusion losses occurred more quickly in the silicone O-ring fitted gas sampling bags for all analytes, except vinyl chloride and 2,5-dimethylfuran, which showed similar loss rates in both bags. These data indicate that replacing the standard silicone O-rings with butyl rubber O-rings will reduce analyte-specific losses from the gas sampling bag over time.

This method reduces adsorption loss on the interior surfaces of the smoking machine by the use of 3 collected clearing puffs and reduces carryover from the remaining residues by performing a series of uncollected clearing puffs before the next smoking run. For the Intense regimen, approximately 75 maximum volume clearing puffs were needed to bring less volatile (e.g., 3-ethyltoluene) residues below the LOD.

Precision. Method precision was evaluated within a single run (intrarun) and among different runs collected on two smoking machines and two GC−MS instruments over several months. For intrarun variability, 14 cigarettes were smoked simultaneously on one smoking machine, which captures differences among cigarettes and in puff engine performance, internal standardization, sample handling, and analysis. For the 3R4F cigarette under ISO conditions, intrarun precision was within 15% RSD for all analytes with the exception of nitromethane (25% RSD). For 3R4F and 1R5F characterizations (N = 137), smoking machine port positions and sample preparation/analysis orders were rotated to randomize these biases among runs. Among-run precisions for 3R4F cigarettes smoked under ISO conditions (Table 2) ranged from 11 to 26% RSD (mean = 18% RSD). The 1R5F data had wider % RSDs ranging from 11 to 38% (mean = 22%). The largest 1R5F variations were observed with nitromethane (38% RSD) and 2,3-butanedione (32% RSD), which were both quantified using peak height. The lesser 1R5F precision is attributed to the higher filter tip ventilation of the 1R5F (70%) compared to the 3R4F cigarette (29%), which reduces VOC delivery and may contribute to cigarette-to-cigarette variability.

Intense regimen data were collected under the same conditions as the ISO regimen sample analyses (N = 106). Under the Intense regimen, RSDs ranged from 8 to 27% (mean = 11%) for 3R4F and from 9 to 29% (mean = 14%) for 1R5F (Table 2). The greater precision of the Intense data set is attributed to the higher VOC deliveries under Intense conditions and added variability associated with filter tip ventilation under ISO conditions.

Methylvinylketone was the least precise analyte in the Intense data set with RSDs of 27% for 3R4F and 29% for 1R5F. These relatively high %RSDs for methylvinylketone are

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Table 3. Comparison of ISO Vapor-Phase Deliveries with Literature Values[a]

	3R4F				2R4F			1R4F			
analyte	This Work Tedlar bag SPME-GC–MS	CORESTA varied methods[b,c] refs 4, 8, 9	Liu 2010 in-line MS ref 14	Uchiyama 2013 sorbent cartridge[d,e] ref 10	Polzin 2007 Tedlar Bag SPME-GC–MS ref 19	Eschner 2011 in-line GC–MS ref 15	Adam 2006 in-line TOF-MS ref 13	Chen 2003 varied methods ref 24	Dong 2004 DNPH GC–MS ref 23	Byrd 1990 Impinger GC–MS ref 5	Hatzinikolaou 2006 in-line GC–UV ref 11
acetaldehyde	620	538[b]	484.6	570[d]		493	310.7	623.88	619.4		626
1,3-butadiene	36.7	38.4[c]	23.6	37[e]		39	17.9	32.10			26.2
furan	23.9					30					20
acrylonitrile	7.70	10.2[c]		8.2[e]				9.51		7.6	
methylvinylketone	34.5				45.7						
butanal	5.81	26.9[b]		26[d]		9		33.93	12.6		
2,3-butanedione	64.1				89.3						
methylethylketone	55.9	48.0[b]		97[d]	86.3	59		68.08	69.9		51
crotonaldehyde	9.51	12.1[b]		15[d]		8		15.90	18.5		15.1
benzene	36.3	39.4[c]	32.8	47[e]	44.1	40	22.5	44.33		45	39.4
2-pentanone	7.48				12.8				5.0		
3-pentanone	3.02				6.1				9.0		
2,5-dimethylfuran	14.3										10.7
toluene	41.1	64.6[c]	49.3	84[e]	57.4	55	37.1	68.08		68	34.8
ethylbenzene	3.34				4.4						
m/*p*-xylene	5.86				9.9						
styrene	1.94	5.0[c]			2.2			6.13		2.1	
o-xylene	1.08				1.7						
3-ethyltoluene	0.438				1.6						

[a]All results are reported in μg/cigarette. [b]Impinger/DNPH derivitization (CORESTA 2010). [c]Various methods (CORESTA 2006 and 2008). [d]DNPH LC–MS. [e]GC–MS.

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attributed to use of a surrogate ISTD rather than an analogue isotopically labeled ISTD.

Accuracy. Method accuracy was evaluated by analysis of third-party reference samples, comparison with literature values (Table 3), and interdependence of VOC levels within and between the two smoking regimens. Third-party reference samples were within 10% of expected values for most analytes. Lower accuracy was observed for compounds that are gases at room temperature and, thus, difficult to gravimetrically measure at room temperature, such as 1,3-butadiene (BP = −4.4 °C). To further evaluate this method's accuracy, the ISO vapor-phase results for the 3R4F cigarettes were compared to literature values (Table 3). Among the 22 analytes, only butanal, toluene, and styrene results differed noticeably from previously reported results. The butanal deliveries, determined here by GC−MS, are lower than results reported by others using LC separation, which is typically not able to separate butanal and isobutanal isomers.[23] Butanal and isobutanal separation was confirmed by a validation mix where the two compounds eluted approximately 1 min apart. Isobutanal delivery was estimated to be 16.2 μg from a 3R4F cigarette using [$^2H_8$]butanal as the ISTD and butanal for calibration. Combining this quantity with that measured for butanal yielded results closer to those reported by liquid chromatography (LC)-MS methods. Nevertheless, the current method's results are still lower than those reported by Dong et al. and Eschner et al., who were able to quantify and separate the butanal isomers using a combination of DNPH derivitization and GC−MS.[15,23]Current toluene results are about 30% lower than previously reported results for 3R4F but show agreement with 2R4F and 1R4F analyses by Adam et al. and Hatzinikolaou et al.[11,13] QC research cigarettes 3R4F, 2R4F and 1R4F have similar design characteristics including tobacco blends and filter tip ventilation; however, statistically significant differences exist between these research cigarette lots.[24] Uchiyama et al., who used a sorbent cartridge for vapor-phase collection,[10] reported higher toluene yields than previous methods. These higher yields may be explained by decreased surface adsorption losses within the smoking apparatus compared to other methods because the sorbent cartridge was installed ahead of the puff engine and it is more common to collect the smoke downstream from the puff engine. Additionally, it is possible that the current method gives lower results than previous methods due to the cleaning of the smoking machine between runs, which both prevents carryover and may also increase adsorption losses. Styrene results agreed with Polzin et al. and Byrd et al., whose methods both used deuterated analogue ISTD for styrene,[5,19] but were lower than Intorp et al. 2009 (CORESTA 2006) and Chen et al.[9,24]

Polzin et al. measured VOCs in cigarette smoke by SPME with gas sampling bag collection and isotopically labeled analogue ISTDs for many compounds. However, unlike the current method, Polzin et al. quantified carbonyl compounds using either [$^2H_6$]acetone or [$^2H_8$]tetrahydrofuran.[19] The current method determined lower deliveries than those reported by Polzin et al. for several carbonyl compounds, probably due to Polzin et al.'s use of a surrogate ISTD for SPME analysis and differences between the 3R4F and 2R4F lots. Specifically, 2-pentanone and 3-pentanone yields were higher using Polzin's method. Although [$^2H_8$]tetrahydrofuran was not used in this analysis, using [$^{13}C_2$]2,5-dimethylfuran as a surrogate ISTD for the two pentanones yielded similar results to Polzin et al. These results indicate the necessity of using analogue ISTDs to correct for analyte-specific matrix effects, SPME fiber competition effects, and loss biases. Polzin et al. reported higher deliveries for 2-butanone, but our results are in agreement with Intorp et al. 2013 (CORESTA 2010), Eschner et al., and Hatzinikolaou et al.[8,11,15]

Pearson correlations were used to evaluate linear dependence between VOC pairs resulting from cigarette-to-cigarette, smoking machine, and sample handling variabilities (Figure 2). Correlations were calculated from only the ISO 3R4F data set, but scatter plots include both the 3R4F and 1R5F ISO data sets. Stronger correlations are expected within compound classes and are indicative of a sensitive and precise method. Correlations were strongest among the monoaromatic compounds (average r = 0.88), with a maximum correlation of r = 0.97 between m/p-xylene and toluene. Styrene was least correlated with the other monoaromatics, but was more highly correlated with the carbonyls than any other monoaromatic. The cyclic ether 2,5-dimethylfuran, which has been used as a smoke exposure biomarker in blood,[1] was well correlated with all compounds except acetaldehyde, 2,3-butanedione, and nitromethane. In general, compounds with lower precision, such as 2,3-butanedione and nitromethane, were less correlated with the other analytes.

	Ethylbenzene (EB)	Styrene (ST)	2,5-dimethylfuran (DF)	2-Pentanone (2P)	Methylethylketone (MK)	Crotonaldehyde (CR)	Butanal (4L)	Nitromethane (NM)
EB	1							
ST	0.88	1						
DF	0.90	0.70	1					
2P	0.81	0.76	0.81	1				
MK	0.79	0.64	0.80	0.89	1			
CR	0.69	0.80	0.72	0.83	0.79	1		
4L	0.50	0.62	0.63	0.82	0.78	0.75	1	
NM	0.33	0.40	0.29	0.36	0.25	0.36	0.41	1

Figure 2. Pearson correlations calculated from VOC deliveries from University of Kentucky research cigarette 3R4F smoked under ISO conditions and scatter plots including both 1R5F (gray) and 3R4F (black) research cigarettes smoked using the ISO regimen.

Butanal correlated well with similar compounds, such as crotonaldehyde (r = 0.75) and methylethylketone (r = 0.78). Between-analyte correlation analysis in a multianalyte method is a useful quality metric tool that can be used to spot trends or outliers that warrant further examination in large, complex data sets. This analysis underscores the importance of simultaneous analyte measurement.

The VOC deliveries of the 1R5F and 3R4F cigarettes smoked under both regimens were compared to further

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evaluate method performance. Assuming that VOC levels are primarily dependent on parameters that contribute to the total puff volume and dilution (i.e., puff number, regimen puff volume and percent tip ventilation), relative VOC concentrations of the research cigarettes and smoking regimens can be estimated and compared to actual results (Table S-1, Supporting Information). The ratios of VOC deliveries for Intense-to-ISO ranged from 6.0 to 24.7 for the 1R5F cigarette (median = 10.4), which are estimated at 5.3. The Intense-to-ISO delivery ratios for the 3R4F cigarette were closer to the estimated ratio of 2.8, ranging from 2.4 to 7.5 (median = 3.4). In general, the deliveries of the more volatile compounds were affected by smoke volume and dilution as expected for regimen differences (Table S-1, Supporting Information). However, less volatile compounds were more affected by the regimen change than estimated. The ratio of VOC deliveries for 1R5F-to-3R4F ranged from 0.18 to 0.38 (median = 0.25) using the ISO regimen, which are estimated at a ratio of 0.31. The 1R5F-to-3R4F delivery ratios using the Intense regimen ranged from 0.42 to 0.98 (median = 0.74) with the estimated ratio being 0.60. Smoke volume and dilution affected 1R5F-to-3R4F relative deliveries similarly to the estimate, although 3R4F values were slightly higher, relative to 1R5Fs, than expected. Relative VOC levels were consistent between the research cigarettes and smoking regimens.

CONCLUSION AND OUTLOOK

The current method has demonstrated accuracy, precision, and high throughput for quantifying 22 toxicologically relevant VOCs in mainstream smoke. Improved throughput compared to previous methods is achieved by automated SPME sampling directly from the gas sampling bags. With over 3000 cigarette brands marketed domestically,[25] this method has sufficient utility to help characterize a representative portion of the market to yield much needed information on exposure ranges typical smokers may encounter. While it has long been known that smoking machine results do not accurately reflect an individual's smoking behavior, our method can capture a wide, dynamic range of deliveries sufficient to characterize upper and lower limits of exposure under naturalistic smoking conditions. In addition, as with any machine-generated smoke analysis, this method provides a means to compare products, design features, and smoking conditions. Mainstream smoke VOC correlations indicate that relative VOC concentrations remain consistent despite cigarette-to-cigarette variability.

ASSOCIATED CONTENT

Supporting Information

Regimen and cigarette design influence on vapor phase concentrations from 1R5F and 3R4F University of Kentucky reference cigarettes smoked under both ISO and Intense regimen conditions, smoking regimen ratio Intense-to-ISO for 1R5F and 3R4F reference cigarettes, and reference cigarette ratio 1R5F-to-3R4F for Intense and ISO regimens. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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