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Quantification of 31 volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography–mass spectrometry ☆

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Abstract

The prevalence of exposure to volatile organic compounds (VOCs) has raised concern about possible health effects resulting from chronic human exposure. To support studies exploring the relation between VOC exposure and health effects, we developed an automated analytical method using solid-phase microextraction (SPME), capillary gas chromatography (GC), and quadrupole mass spectrometry (MS). This method quantifies trace levels (low parts per trillion) of 14 halogenated alkanes, 5 halogenated alkenes, 10 aromatic compounds, and 2 other VOCs in human blood. Detection limits for the SPME–GC–MS method range from 0.005 to 0.12 μg/L, with linear calibration curves spanning three orders of magnitude. The improved throughput of this method will enable us to expand biomonitoring efforts to assess nonoccupational VOC exposure in large epidemiological studies.
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Keywords: Volatile organic compound; VOC; Solid-phase microextraction; Mass spectrometry; Human; Whole blood

1. Introduction

Volatile organic compounds (VOCs) originate from many different natural and anthropogenic sources and exist in virtually all homes and workplaces. Common VOC exposure sources include tobacco smoke, petroleum products, and chlorinated water. The resulting ubiquitous exposure to VOCs is an area of public health concern [1]. Long-term exposure to VOCs may increase risk for leukemia [2], bladder cancer [3], birth defects [4], and neurocognitive impairment [5]. The widespread and ongoing exposure to these potentially harmful chemicals merits further research, including development of improved exposure assessment methods. Variables in human absorption, distribution, metabolism, and excretion of environmental toxicants complicate estimation of exposure to VOCs. Therefore, measuring internal dose is often the best method for assessing the extent to which human exposure to environmental toxicants, including VOCs, may lead to adverse health outcomes [6].

Previous methods for quantifying VOC exposure have focused on measuring VOCs (and VOC metabolites) in breath, blood, and urine. Analysis of urinary VOC metabolites is useful for assessing occupational exposure to some VOCs (e.g., benzene, styrene, and tetrachloroethene) but has yet to be tested broadly for evaluating exposure at background levels. If the VOCs themselves are measured in urine, during collection they may diffuse from the warm salty aqueous matrix into the atmosphere, resulting in potential underestimation of actual exposure. After significant exposure, VOCs can be detected in exhaled breath [7–10]. Measurement of VOCs in breath is noninvasive and provides complimentary information to measurement of VOCs in blood. Assessing VOCs in blood may yield more complete data sets on a matrix that is closer to the target tissue(s) [11,12].

Previous methods for quantifying VOCs in blood lacked either sensitivity, ruggedness, throughput, or broad applicability [13–16]. Therefore, we developed an improved method for quantifying 31 target VOCs in 3 mL of whole blood using headspace solid-phase microextraction (SPME) coupled with capillary gas chromatography (GC) and quadrupole mass spectrometry (MS).

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Table 1
Analytical parameters for the determination of selected VOCs.

Retention time (min)	Analyte	Quant[a] (*m/z*)	Conf[b] (*m/z*)	IntStd[c] label	IntStd (*m/z*)	Dwell (ms)	Reportable range (μg/L)
8.48	1,1-Dichloroethene	96	98	$^{2}H_2$	100	30	0.009–8.1
8.78	Methylene chloride	84	49	$^{13}C_1$	85	30	0.070–18
9.85	*trans*-1,2-Dichloroethene	96	98	$^{2}H_2$	100	25	0.009–9.1
10.07	Methyl *tert*-butyl ether	73	57	$^{2}H_{12}$	75	25	0.100–20
10.23	1,1-Dichloroethane	63	65	$^{2}H_3$	66	25	0.010–4.7
11.13	*cis*-1,2-Dichloroethene	96	98	$^{2}H_2$	100	30	0.010–9.3
11.46	Chloroform	83	85	$^{13}C_1$	86	30	0.020–9.0
12.45	1,2-Dichloroethane	62	64	$^{2}H_4$	67	30	0.009–9.3
12.58	1,1,1-Trichloroethane	97	99	$^{2}H_3$	102	30	0.048–23
13.13	Carbon tetrachloride	117	119	$^{13}C_1$	120	30	0.005–4.9
13.21	Benzene	78	77	$^{13}C_6$	84	30	0.024–12
14.09	Dibromomethane	174	93	$^{2}H_2$	178	15	0.030–18
14.16	1,2-Dichloropropane	63	76	$^{2}H_6$	67	15	0.008–7.3
14.23	Trichloroethene	130	132	$^{13}C_1$	133	15	0.012–5.6
14.31	Bromodichloromethane	83	85	$^{13}C_1$	86	15	0.030–12
15.54	2,5-Dimethylfuran	96	95	$^{13}C_1$	98	60	0.012–11
16.35	1,1,2-Trichloroethane	97	83	$^{2}H_3$	102	60	0.010–7.4
16.66	Toluene	91	92	$^{13}C_7$	98	60	0.025–12
17.16	Dibromochloromethane	129	127	$^{13}C_1$	130	60	0.005–4.6
17.89	Tetrachloroethene	166	164	$^{13}C_1$	169	60	0.048–22
19.08	Chlorobenzene	112	77	$^{13}C_6$	118	60	0.011–4.7
19.47	Ethylbenzene	91	106	$^{13}C_6$	97	30	0.024–12
19.83	*m*/*p*-Xylene	91	106	$^{13}C_6$	97	30	0.034–16
19.93	Bromoform	173	175	$^{13}C_1$	174	30	0.020–20
20.42	Styrene	104	103	$^{13}C_6$	110	20	0.050–26
20.54	1,1,2,2-Tetrachloroethane	83	85	$^{2}H_2$	86	20	0.010–4.8
20.54	*o*-Xylene	91	106	$^{13}C_6$	112	20	0.024–6.1
23.42	1,2-Dichlorobenzene	146	111	$^{2}H_4$	150	60	0.100–6.5
22.97	1,3-Dichlorobenzene	146	111	$^{13}C_6$	152	60	0.050–7.4
23.05	1,4-Dichlorobenzene	146	111	$^{13}C_6$	152	60	0.120–38
23.94	Hexachloroethane	201	166	$^{13}C_1$	204	60	0.011–5.8

[a] Quantification ion.
[b] Confirmation ion.
[c] Internal standard.

This method achieves the selectivity and sensitivity needed to measure VOCs in human blood. Additionally, the improved ruggedness and throughput of this method enables cost-effective quantification of VOCs of interest in the general population.

2. Experimental

2.1. Materials

Purge-and-trap–grade methanol purchased from Burdick and Jackson (Muskegon, MI) was used to prepare all standards and to rinse glassware. HPLC-grade water was purchased from J.T. Baker (Phillipsburg, NJ). Water typically is contaminated with variable trace levels of VOCs, especially chloroform; therefore, source water was further purified using helium purging and distillation [17]. Because water purity varies widely between production lots, a sufficient quantity of water was distilled so the same lot would be used to prepare all solutions, blanks, standards, and quality control (QC) material during these experiments. Stainless steel needles (18-gauge, Luer-Lok) were purchased from Becton Dickinson (Franklin Lakes, NJ). Reaction vials (10 mL, serum type) were purchased from Microliter Inc. (Suwanee, GA). Septa (20 mm, Teflon-faced/silicone), seals (aluminum, open center), SPME fibers (75 μm Carboxen/PDMS) and SPME–GC inlet liners (0.75 mm I.D.) were purchased from Supelco (Bellefonte, PA). Chromatographic separation was performed on a 40 m × 0.18 mm × 1 μm DB-VRX column (Agilent Technologies, Palo Alto, CA).

2.2. Standards

All analytes and internal standards (Table 1) were purchased as neat compounds of the highest purity available and stored at −20 °C to minimize degradation. Stable isotope-labeled analogs were primarily ^{13}C-labeled to better mimic the partitioning, chromatography, ionization, and mass analysis of the native compounds. Standards and labeled analogs were prepared from neat chemicals and diluted with methanol (purge-and-trap–grade) to intermediate stock solution concentrations. These stock solutions were sealed in glass ampoules and stored at −70 °C. On the day of use, stock solutions of standards were diluted further in helium-sparged/distilled water. Solutions of labeled analogs were prepared by further dilution with methanol (purge-and-trap–grade) and 40.0 μL was added to 3-mL quantities of each blank, standard, unknown, or QC sample being analyzed. Positive displacement pipettes and glass capillary

tips were used for all liquid transfers in the microliter range [14].

2.3. Blood collection vial preparation

Blood samples for VOC analysis were collected into specially treated blood collection vials (Vacutainers®, Becton-Dickinson, Franklin Lakes, NJ). Depending on the needs of the study either 7-mL (13 mm × 100 mm) or 10-mL (16 mm × 100 mm) draw Vacutainers were used. These gray-top Vacutainers contain potassium oxalate and sodium fluoride to prevent clotting and inhibit cellular metabolism. To remove VOC residue from the Vacutainer stoppers, the Vacutainers were disassembled and the butyl rubber stoppers heated for 17 days at 80 °C in a vacuum oven (∼100 kPa) as described by Cardinali et al. [18]. One day before Vacutainer reassembly the Vacutainer vials also were baked at 80 °C in a vacuum oven (∼100 kPa). After the ovens cooled to room temperature, ultra high-purity nitrogen was used to equilibrate the ovens to atmospheric pressure. The Vacutainers then were reassembled and vacuum redrawn through a small-gauge needle (1/2-in., aluminum luer lock hub, 27-gauge, Sherwood Medical; St. Louis, MO). Once vacuum was reestablished, the Vacutainers were sterilized using a GammaCell 220 Irradiator (AECL; Kanata, Ont., Canada) with a Cobalt-60 source. Vacutainers were dosed with approximately 250,000 rads to inactivate any microbes that might have been deposited within the Vacutainer during processing. Vacutainers treated by this protocol have a shelf life of approximately 1 year.

2.4. Blood sample collection

Blood samples were collected by venipuncture as described by Ashley et al. [14]. Vacutainers were filled completely to minimize headspace. Care was taken to fully dissolve the potassium oxalate/sodium fluoride powder in each tube immediately after blood collection and thus minimize clotting. These samples subsequently were stored chilled (4 °C) in the dark until analysis within 10 weeks after sample collection.

2.5. Blood sample analysis using SPME

Blood Vacutainers were removed from refrigerated storage (4 °C) and allowed to equilibrate to room temperature while being mixed with a hemo-mixer for at least 30 min before sample preparation. Subsequently, blood (3.0 mL) was removed using a precleaned gas-tight syringe (5 mL, glass, Unimetrics, Shorewood, IL) and transferred into a SPME headspace vial. The sample weight was gravimetrically determined because of the relative imprecision of liquid measurement of whole blood. Labeled analog solution (40.0 μL) was added to the sample using a positive displacement pipette (VWR Scientific, West Chester, PA) and the SPME vial immediately crimp-sealed using a Teflon-lined septum and steel/aluminum crimp seal. Blanks, standards, and QC samples were similarly processed. Samples were prepared and analyzed in daily batches of 28 unknowns, 7 calibrators, 4 QC specimens, and 1 blank.

2.6. Instrumentation

The high-throughput SPME–GC–MS method was developed on an Agilent Technologies 6890A gas chromatograph–5973 N mass spectrometer (GC–MSD, Agilent Technologies, Palo Alto, CA) equipped with a split/splitless injector operated in the pulsed splitless mode using a narrow-diameter liner specially designed for SPME sample introduction. The GC–MSD system was controlled by Agilent Technologies ChemStation (G1701CA) for data acquisition. A cryotrap (model 961, Scientific Instrument Services, Ringoes, NJ) initially cryofocused VOCs in approximately the first 11 cm of the GC column during thermal desorption of the SPME fiber. With liquid nitrogen coolant, the cryotrap was maintained at −100 °C for the first 1.5 min of analysis and then heated ballistically to 225 °C to desorb the analytes from the front of the column. The rest of the GC column was maintained at an initial oven temperature of 0 °C using liquid nitrogen coolant to further focus the volatile analyte band. An inlet pressure pulse of 50 psi was maintained for 1.5 min along with the initial oven temperature and the cryotrap trapping temperature. Helium (research grade, 99.9999%, Airgas South, Atlanta, GA) served as the carrier gas with a constant flow of 1.0 mL/min (except for the initial 1.5 min pressure pulse). The GC was held at the initial temperature of 0 °C for 1.50 min followed by a linear thermal gradient of 7 °C/min to 140 °C then ramped at 40 °C/min to 220 °C and held for 4.5 min resulting in a run time of 28 min. Automated sampling was done using a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) equipped with a 75-μm Carboxen®/polydimethylsiloxane (PDMS) SPME fiber assembly. After preparation, samples were queued in a Peltier cooled rack (15 ± 1 °C) before analysis. Sample analysis was initiated by moving the active sample to a heated agitator station (40 °C). Following pre-incubation (10 s) the SPME fiber was inserted into the vial headspace and the sample extracted (6 min, 500 rpm). The fiber was promptly sheathed and transferred into the GC inlet where VOCs were desorbed in the hot inlet (200 °C). The SPME fiber remained in the GC inlet for the remainder of the GC run to ensure complete analyte desorption and to minimize contamination from laboratory air.

The mass spectrometer was equipped with an electron ionization source and operated in the selected ion monitoring (SIM) mode tuned to nominal unit mass resolution and using the "high resolution" option. For each analyte the mass spectrometer was set to monitor three ions: one each for quantification, confirmation of identity, and the isotopically labeled internal standard. Quantification ions were selected as the most abundant ion in the mass spectrum that did not compromise the specificity of the analysis. The retention time and mass apex for each compound was determined by analyzing known standards in full scan mode and adjusting the SIM acquisition conditions accordingly. Multiple SIM acquisition groups were created to maximize dwell time and sensitivity by minimizing the number of compounds analyzed in any given time frame. Chromatographic peak widths were approximately 4 s, baseline to baseline; and SIM dwell times were adjusted to provide 15–20 data points per analyte in each SIM group. SPME of the next sample in the run queue

began at the end of the GC run (before cryocooling of the GC inlet and oven) to minimize overall cycle time (37 min).

2.7. Quantification

Xcalibur Quan software (ThermoFinnigan, San Jose, CA) was used for peak integration, calibration, and quantification. Data were exported from Chemstation software into AIA/NetCDF format. Subsequently these CDF files were converted to Xcalibur RAW format using the Xconvert feature of the Xcalibur software. These translated data then were imported into Xcalibur Quan for further examination and processing. Peak integrations were confirmed by visual inspection. Relative response ratios were calculated on the basis of the relative peak areas of analyte quantification ion and labeled analog ion. The set of seven calibrators analyzed with each set of samples generated the calibration curve for that day. These linear calibration curves (r^2 typically $\geq$0.99) spanned three orders of magnitude. Calibration curves were adjusted for ion cross-contamination between native analyte and isotopic analog using a feature similar to the correction described by Colby and McCaman [19]. The lowest calibrators ranged from 5 to 120 pg/mL. The limit of detection (LOD) was three times the standard deviation at zero concentration (S_0). S_0 was determined by analyzing at least six sets of the lowest four calibration standards and plotting the standard deviation versus the known standard concentration. The *y*-intercept of the best-fit line of this plot was used as S_0. The calculated value for $3S_0$ typically was lower than the lowest standard and thus the lowest reportable value for each analyte typically equaled the lowest standard level [20]. Samples with analyte concentrations above the highest calibrator were reanalyzed using a reduced sample volume.

2.8. Quality assurance

Data were subjected to QC procedures using a custom laboratory information management system constructed in Microsoft Access. Contamination was evaluated both qualitatively and quantitatively. Laboratory air was extracted using SPME for 6 min and subsequently analyzed by SPME–GC–MS as described above. The resulting chromatograms were qualitatively reviewed for gross contamination of VOCs. Furthermore, analysis of a VOC-free water blank identified any analyte contamination that might bias results.

After analyzing samples and visually inspecting every integrated peak, we evaluated additional QC parameters. Adequate labeled analog response was evaluated on the basis of absolute peak area signal and signal-to-noise ratio. We further evaluated the identity of the analyte ion by comparing the confirmation ion ratio in unknown samples with that for reference standards. Each batch of data was evaluated against blind QC samples.

2.9. Quality control samples

Four QC samples were processed and analyzed with each batch of samples. These samples were prepared by spiking concentrated standards into bovine serum and equilibrating the VOCs in this mixture. Subsequently aliquots were stored at −70 °C in flame-sealed glass ampoules. On the day of use, an aliquot of QC serum was sampled as though it were an unknown. An independent QC officer evaluated blind QC samples according to modified Westgard QC rules [21] Assay precision was characterized for each analyte based on at least 15 separate determinations. QC failed for an analyte if results deviated from characterized means by three standard deviations, two consecutive determinations exceeded two standard deviations, or ten consecutive determinations fell on the same side of the mean. If QC sample results failed for an analyte, then all results for that analyte on that day/batch were rejected.

2.10. Blank analysis

Trace levels of VOCs such as methylene chloride, chloroform, trimethylsilanol, benzene, toluene, xylenes and methyl-*tert*-butyl ether (MTBE) are ubiquitous in a typical laboratory; rigorous technique is required to minimize sample contamination from laboratory air. Potential sources of contamination include chlorinated water, common household cleaning products, laboratory solvent use, and exhaust from oxygenated fuel use contaminating the source of laboratory air. Volatile contaminants from these and other sources easily can spread through laboratory air to samples during preparation (sample handling) or analysis (SPME fiber). Contamination was minimized by removing sources of VOCs from the laboratories (where possible). A blank water sample was used to test for contamination. Blank water was prepared by helium sparging, distilling, and flame sealing in glass ampoules. On the day of use, a water blank was spiked with labeled internal standards and run with each batch of unknowns. If the blank contained analyte levels exceeding the LOD, then the run was flagged as contaminated for that analyte. Additionally, a SPME fiber sampling of laboratory air was run to qualitatively assess airborne contaminants.

2.11. Proficiency testing

Proficiency testing materials were prepared from a commercially available VOC mixture (Environmental Protection Agency mix 524 rev A, Supelco). Additional analytes not found in this VOC mixture (e.g., 2,5-dimethylfuran) were fortified with gravimetrically confirmed amounts of neat material. Individual proficiency testing pools were prepared by serial dilution into methanol. Each of these proficiency testing pools were aliquotted into glass ampoules and flame sealed. These ampoules then were blind-coded by an independent QC officer and five ampoules analyzed in blinded fashion every 6 months or after major instrument maintenance. On the day of analysis, proficiency testing materials were diluted into distilled water and analyzed. The assay passed proficiency testing if blind analyzed amounts fell within 25% of actual values.

3. Results and discussion

Our previous methods for quantifying alkanes, alkenes, aromatics and ethers in blood employed purge-and-trap extraction

followed by high-resolution MS [14]. The accuracy, selectivity and sensitivity of this approach were excellent, but the method did not apply broadly to large epidemiologic studies because of poor throughput (eight unknowns per day), relative imprecision (average relative standard deviation of 22%), and high cost ($400 per unknown). The SPME–GC–MS method presented here improved throughput four-fold while cutting overall cost of analysis in half and producing data with better precision (average relative standard deviation of 9%) compared with our previous method for quantifying alkanes, alkenes, aromatics and ethers in human blood. This method builds on other published work using SPME to extract VOCs from aqueous matrices [15,16]. To achieve the necessary selectivity and sensitivity for VOC measurements in human blood while using unit mass resolution MS, we had to address issues with interferences and LODs that were not problematic for the high-resolution MS method.

Given the decrease in mass spectral resolution resulting from the use of quadrupole MS, adequate chromatographic resolution became crucial. Several analytes produced fragment ions of similar mass/charge ratio, and thus could not be resolved at unit mass resolution, so changes to the chromatography were used to separate interferences from the analytes of interest. A previous method developed in our laboratory for a more limited set of analytes [16] employed a 30 m × 0.32 mm × 1.2 μm DB-624 column. We improved column efficiency and resolved flow problems by using a column of smaller diameter (0.18 mm). Experiments with a 40 m Restek VRX column demonstrated better overall separation for the 31 analytes but with less than baseline chromatographic resolution of benzene and carbon tetrachloride. Although we observed no isobaric interferences with the native analytes, the molecular ion for the $^{13}C_6$-labeled benzene had an isobaric interference with the $C^{35}Cl^{37}Cl^+$ fragment ion from the native carbon tetrachloride. The potential interference of an ion from native carbon tetrachloride with the internal standard for benzene required baseline chromatographic resolution between these two analytes. We found that the Agilent Technologies DB-VRX gave baseline resolution for the native and labeled carbon tetrachloride and benzene, while maintaining all other critical separations.

A similar problem that existed with the separation of *o*-xylene and 1,1,2,2-tetrachloroethane, where the $^{13}C_6H_5^+$ fragment ion was isobaric at *m/z* 83 with the $CH^{35}Cl_2^+$ fragment, was corrected by choosing a different labeling scheme of the internal reference compound. The VRX column demonstrated insufficient chromatographic resolution to separate this pair of compounds. Rather than explore alternative stationary phases, the internal standard for the *o*-xylene was changed from the $^{13}C_6{}^{12}C_2H_{10}$ to $C_8H_4{}^2H_6$ resulting in a decreased retention time for the xylene fragment and changing the *m/z* from 83 to 112 ($C_8H_4{}^2H_6^+$) for which no significant fragment existed for the 1,1,2,2-tetrachloroethane. Each of the three isomers of xylene and dichlorobenzene produced indistinguishable spectra and required chromatographic resolution for separate reporting. All of these isomers were chromatographically resolved except for *m*-xylene and *p*-xylene, which are therefore reported as a combined value, as has been done previously. Fig. 1 shows GC traces resulting from the analysis of a blood sample for benzene.

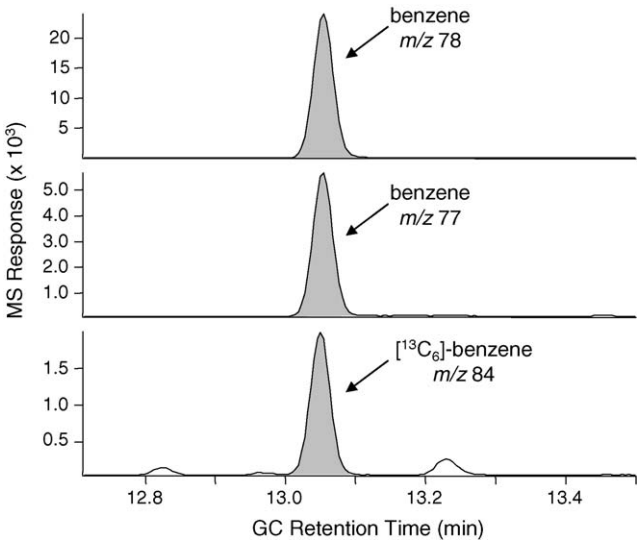

Fig. 1. Typical chromatogram resulting from the analysis of benzene in human blood.

Note that the quantification (*m/z* 78), confirmation (*m/z* 77) and labeled analog (*m/z* 84) ions are well resolved from potential interfering compounds. These data are typical of the analysis of the other VOCs and results from the combination of a selective detector and efficient chromatographic separation.

Some of the 31 VOC analytes had potential interferences from compounds that were not in the sample originally but resulted from the sample collection or measurement process. For example, trimethylsilanol, which is ubiquitous in the laboratory environment and is easily extracted from air or water onto a Carboxen/PDMS SPME fiber, can interfere with MTBE. Trimethylsilanol elutes a few seconds before MTBE and fragments to produce a large *m/z* 75 ion signal. The $^{13}C_3$ analog of MTBE also fragments to produce a base peak at *m/z* 75. The large tailing trimethylsilanol peak at *m/z* 75 can fully conceal the $^{13}C_3$-MTBE peak and thus prevent accurate quantification of MTBE in the sample. Materials such as Teflon-faced/silicone rubber septa can be contaminated with trimethylsilanol. To minimize interference from trimethylsilanol, the $^{13}C_3$ analog of MTBE was replaced by $^2H_{12}$ MTBE in this assay, changing the mass analyzed and removing the interference.

Headspace extraction using the Carboxen/PDMS SPME fiber presented a particular challenge for trace VOC analysis. The sorbent matrix can deteriorate resulting in both loss of absolute collection efficiency and formation of decomposition byproducts that can interfere with analyses. For the Carboxen/PDMS SPME fiber, the PDMS adhesive not only can be a source of siloxane congeners but also siloxane side groups that can include methane, ethane, benzene, and toluene [22]. Formation of these byproducts became apparent after repeated sampling of the same water blank suggested the source of the aromatic hydrocarbons was neither carryover from previous samples nor environmental contamination. By using a programmable temperature vaporizing (PTV) inlet, we determined that benzene and toluene were off-gassing from the SPME fiber at a constant rate. When the fiber was removed from the inlet and reinserted imme-

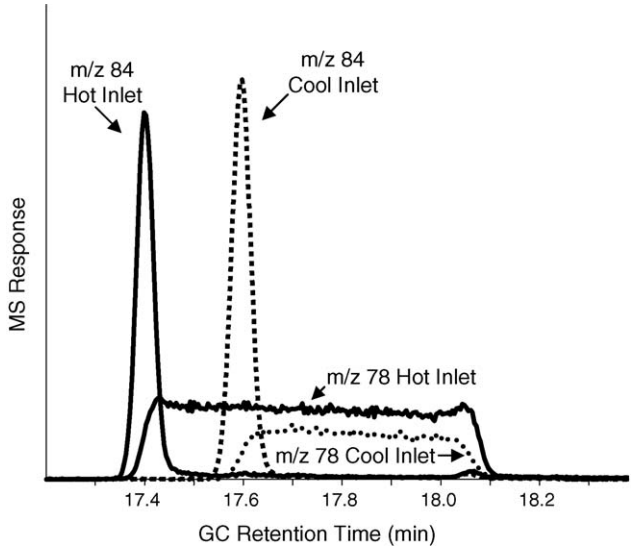

Fig. 2. Differentiation of benzene desorption from Carboxen/PDMS SPME fiber by using single stage cryotrapping and varied inlet temperature. Higher levels of benzene desorb from the SPME fiber in the hotter GC inlet (solid line) compared with the cooler GC inlet (dashed line).

diately, low-level signals for both benzene and toluene still were observed, but if no fiber was in the fiber holder, no signal was observed. To identify the source of the benzene and toluene contamination, we used single-stage cryotrapping and varied inlet temperature after headspace extraction of a sample containing $^{13}C_6$-benzene internal standard (Fig. 2). The $^{13}C_6$-benzene from the sample headspace produced the expected Gaussian peaks of similar magnitude at inlet temperatures of 200 °C and 250 °C. Conversely, the benzene contaminant produced an asymmetrical nongaussian peak that increased in magnitude with increased inlet temperature. This shows that the benzene contaminant observed at *m/z* 78 was not produced by the same process as the $^{13}C_6$-benzene at *m/z* 84. Results were similar for toluene. We hypothesize that the benzene and toluene contaminants resulted from the continuing degradation of the SPME fiber. Although the manufacturer has not confirmed this, we hypothesize that the SPME fiber sorbent degrades under our sampling conditions to produce small amounts of benzene and toluene subsequently desorbed into the inlet. Attempts to use alternative fiber sorbents resulted in poor sensitivity for the most volatile compounds. Therefore the Carboxen®/PDMS fiber was retained, recognizing the potential problems associated with trace levels of benzene and toluene presumably produced by the SPME itself. By decreasing the inlet temperature to 200 °C and the purge activation time to 1.5 min, we reduced the amount of benzene and toluene contamination from fiber degradation and bleed. Blank samples analyzed with this method yielded benzene and toluene levels less than the method LOD.

Because of the reuse of the fiber, SPME presents carryover problems not present in purge and trap. Despite the wide range of VOC concentrations measured in human samples (0.005–160 μg/L) most analytes did not have significant problems with carryover of analyte from one sample to the next. However, the aromatic analytes, especially 1,4-dichlorobenzene, presented some carryover issues. The carryover limits for these analytes are defined as the maximum levels that can be analyzed without carrying over into the next sample above the LOD (Table 2). Blood samples containing 1,4-dichlorobenzene above 12.1 μg/L left a significant residual on the fiber assembly, which then could be desorbed during analysis of the next sample. Exposure to 1,4-dichorobenzene through consumer products can lead to blood levels of this VOC that exceed the carryover limit [23]. When this occurs, the sample with elevated levels of 1,4-dichlorobenzene must be reanalyzed using a reduced volume. Additionally, the blood sample analyzed immediately after the sample with the high level must be reanalyzed. We found the best way to reduce both contamination and carryover from the SPME fiber was to leave the fiber in the heated GC inlet during the entire analysis. This heated zone is purged with helium at 50 mL/min (split mode), leading to more effective removal of all aromatic components from the fiber assembly than with the CTC CombiPal fiber conditioning station.

Table 2
Carryover limits for VOC analytes

Analyte	Carryover limits (μg/L)
1,2-Dichlorobenzene	2.3
1,3-Dichlorobenzene	2.0
1,4-Dichlorobenzene	12.1
Benzene	3.8
Chlorobenzene	1.5
Ethylbenzene	3.8
m/p-xylene	5.1
o-Xylene	1.9
Styrene	8.3
Toluene	3.9

Contamination presents another potential problem area during quantification of VOCs in human blood. Some VOC analytes are more prone than others to contamination. VOC contamination can derive from many sources, including laboratory air [16]. VOCs can contaminate samples when permanent ink markers (Sharpie, Sanford Inc., Bellwood, IL) are used to label SPME bottles during sample preparation. Specifically, we identified contamination of benzene, toluene, *o/m/p*-xylenes, chlorobenzene, ethylbenzene, and nitrobenzene offgassing from the markers. The common use of organic solvents such as methylene chloride in analytical laboratories also can contaminate samples. Consumable materials that closely contact the sample are potential sources of contamination. In the past, we found butyl rubber Vacutainer stoppers were contaminated with nanogram quantities of bromoform, 1,4-dichlorobenzene, dibromomethane, ethylbenzene, benzene, xylenes, toluene, and trichloroethene [18]. Additionally, use of certain polysiloxane curing agents in the manufacture of sample vial septa can lead to the formation of microgram quantities of MTBE that subsequently can contaminate samples [24]. Use of only pretreated and/or prescreened materials and reagents generally can prevent contamination.

Even after pretreatment to remove residual VOCs from materials, prescreening is advised before use of the materials. For example, two different lots of Becton-Dickinson blood

collection tubes were pretreated to remove VOC contaminants as described previously by Cardinali et al. [18]. Possibly because of subtle differences during manufacturing, Vacutainers from one lot still contained unacceptable amounts of benzene after pretreatment, leading to measurable benzene in blank samples stored in the Vacutainers from this lot for 6 days (0.060 ± 0.002 μg/L) and 36 days (0.082 ± 0.005 μg/L), whereas identical sample stored in Vacutainers from another lot contained no measurable levels of benzene. As an additional effort to identify systematic contamination problems, we analyzed a method blank along with each batch of unknown samples. If this blank indicated analyte contamination in excess of the method LOD, then the analysis batch was rejected for that analyte.

The SPME–GC–MS method produced excellent LODs for most VOC analytes as seen in the lower reportable limit (Table 1). Analysis of blood samples containing each VOC analyte at the lowest reportable value typically produced a quantitation ion peak with a signal-to-noise ratio exceeding 15:1. The LODs for trihalomethanes and MTBE using this low resolution MS method were 8–100-fold poorer than LODs produced with a method using high resolution MS to measure only these five analytes [15]. The advantage of using low resolution quadrupole MS is that the instrumentation is less expensive and can scan for a broad range of analyte ions without concerns about magnetic hysteresis.

Levels of VOCs in unknown samples were quantified against calibrators ranging from 0.005 to 38 μg/L (Table 1). A broad linear range was required because of highly variable VOC levels in human blood specimens; previous human blood data indicate VOC levels can vary by over three orders of magnitude between different people [1]. Each batch of unknown samples was analyzed with a set of seven calibrators (one at each of seven different concentrations), and the relative response of those calibrators used to draw the calibration curve for that particular day. Calibration curves are weighted with the inverse of the concentration and typically have correlation coefficients of >0.99, with excellent linearity across the calibration range, including at lower concentrations. Repeated analysis of calibrators at each concentration level produced reproducible intraday results (e.g., benzene: 0.037 μg/L, relative standard deviation (R.S.D.) = 11.7%; 11.9 μg/L R.S.D. = 0.3%), reinforcing the precision of this daily calibration procedure. Interday variability of daily calibration curve slopes was significant; the R.S.D. of 20 calibration curve slopes analyzed over an 8-week period was 4.7%. Slight differences in response ratios also were observed when identical sets

Table 3
Reproducibility of analysis of VOCs in quality control sera over a two year period ($n > 120$).

Analyte	Low QC[a] mean ± S.D.[b] (μg/L)	R.S.D.[c] (%)	High QC mean ± S.D. (μg/L)	R.S.D. (%)
1,1,1-Trichloroethane	0.086 ± 0.011	13	0.436 ± 0.055	13
1,1,2,2-Tetrachloroethane	0.028 ± 0.002	6	0.135 ± 0.005	4
1,1,2-Trichloroethane	0.042 ± 0.002	5	0.207 ± 0.007	3
1,1-Dichloroethane	0.021 ± 0.002	11	0.105 ± 0.009	9
1,1-Dichloroethene	0.026 ± 0.003	12	0.138 ± 0.025	18
1,2-Dichlorobenzene	0.050 ± 0.006	13	0.216 ± 0.012	6
1,2-Dichloroethane	0.052 ± 0.005	9	0.253 ± 0.014	6
1,2-Dichloropropane	0.037 ± 0.003	8	0.181 ± 0.011	6
1,3-Dichlorobenzene	0.053 ± 0.014	26	0.204 ± 0.017	8
1,4-Dichlorobenzene	0.264 ± 0.029	11	1.13 ± 0.054	5
2,5-Dimethylfuran	0.064 ± 0.006	9	0.318 ± 0.027	8
Benzene	0.064 ± 0.010	16	0.293 ± 0.024	8
Bromodichloromethane	0.065 ± 0.004	6	0.320 ± 0.013	4
Bromoform	0.121 ± 0.005	4	0.577 ± 0.016	3
Carbon tetrachloride	0.017 ± 0.003	19	0.087 ± 0.015	17
Chlorobenzene	0.028 ± 0.002	7	0.134 ± 0.006	4
Chloroform	0.058 ± 0.013	23	0.230 ± 0.016	7
cis-1,2-Dichloroethene	0.045 ± 0.004	9	0.221 ± 0.019	9
Dibromochloromethane	0.027 ± 0.001	5	0.130 ± 0.004	3
Dibromomethane	0.102 ± 0.008	8	0.497 ± 0.025	5
Ethylbenzene	0.212 ± 0.017	8	0.484 ± 0.025	5
Hexachloroethane	0.034 ± 0.002	7	0.166 ± 0.010	6
m/*p*-Xylene	2.24 ± 0.147	7	2.78 ± 0.153	6
Methylene chloride	0.156 ± 0.053	34	0.483 ± 0.057	12
o-Xylene	0.242 ± 0.016	7	0.381 ± 0.030	8
Styrene	0.200 ± 0.023	11	0.779 ± 0.035	5
Methyl *tert*-butyl ether	0.115 ± 0.008	7	0.568 ± 0.027	5
Tetrachloroethene	0.201 ± 0.027	13	0.809 ± 0.105	13
Toluene	0.329 ± 0.041	12	0.591 ± 0.048	8
trans-1,2-Dichloroethene	0.039 ± 0.005	12	0.198 ± 0.025	13
Trichloroethene	0.038 ± 0.004	11	0.161 ± 0.017	11

[a] Quality control serum.
[b] Standard deviation.
[c] Relative standard deviation.

of calibrators were analyzed on three similarly configured instruments. To validate the use of aqueous calibrators for quantifying VOCs in blood samples, we fortified a set of identical blood samples with calibration standards. The resulting data were used to draw a calibration curve in blood for each analyte; the slopes of calibration curves prepared in blood did not differ from the slopes of calibration curves prepared in water. On the basis of this variability between days and instruments, we recommend quantifying unknown samples using calibrators analyzed within the same batch and on the same instrument. These individual calibration curves help to adjust for interday variation and result in improved long-term precision of the assay. The day-to-day reproducibility of QC samples analyzed repeatedly improved significantly using daily calibrations. Daily analysis of a full set of calibrators was not possible using our previous purge and trap approach and was made possible by the improved throughput of the SPME–GC–MS method.

Multiple analyses of QC materials were used to evaluate the precision of the assay. Each mean and standard deviation (Table 3) derives from at least 120 individual data points acquired from repetitive analyses of QC serum during a 2-year period on three similarly configured, but separate instruments. The QC serum contained concentrations of individual VOCs ranging from 0.017 to 2.78 μg/L, similar to the range of VOC concentrations found in unknown samples. The levels of *m*/*p*-xylene were significantly higher than in blood, and resulted from contamination in the bovine serum used for formulating the QC pools. All but three of the relative standard deviations were <20% for all analytes and levels. The methylene chloride low concentration material presented the poorest reproducibility, probably because of the variable contamination of the laboratory environment discussed above. The precision of methylene chloride detection at higher concentrations was much better (R.S.D. = 12%). As would be expected, the relative analytical precision was better for the QC pool containing higher analyte levels (mean R.S.D. = 7%), compared with QC pool at lower analyte levels (mean R.S.D. = 11%). The precision with this assay is significantly better than with our previous high-resolution full-scan MS method, because of faster scanning rates producing 15–20 scans per peak.

Accuracy is a significant concern when moving from a more selective to a less selective method. To evaluate any loss in selectivity, every 6 months we evaluated certified reference material diluted to various proficiency testing concentrations. Blind

Table 4
Method accuracy based on analysis of VOCs in proficiency testing materials (μg/L)

Analyte	PT[a] level 1		PT level 2		PT level 3		PT level 4	
	Mean ± S.D.[b]	Diff[c] (%)	Mean ± S.D.	Diff (%)	Mean ± S.D.	Diff (%)	Mean ± S.D.	Diff (%)
1,1,1-Trichloroethane	0.48 ± 0.06	0	0.95 ± 0.04	0	1.82 ± 0.04	−4	3.25 ± 0.12	−17
1,1,2,2-Tetrachloroethane	0.34 ± 0.01	5	0.68 ± 0.02	7	1.40 ± 0.03	9	3.19 ± 0.15	24
1,1,2-Trichloroethane	0.30 ± 0.01	−5	0.60 ± 0.01	−7	1.15 ± 0.02	−10	2.31 ± 0.11	−10
1,1-Dichloroethane	0.28 ± 0.03	−12	0.56 ± 0.02	−13	1.08 ± 0.02	−16	2.15 ± 0.06	−16
1,1-Dichloroethene	0.24 ± 0.03	−24	0.48 ± 0.02	−25	1.02 ± 0.04	−20	2.14 ± 0.04	−17
1,2 Dichlorobenzene	0.32 ± 0.02	1	0.62 ± 0.01	−3	1.19 ± 0.04	−7	2.34 ± 0.1	−9
1,2-Dichloroethane	0.32 ± 0.02	−2	0.62 ± 0.02	−3	1.20 ± 0.03	−6	2.44 ± 0.13	−5
1,2-Dichloropropane	0.29 ± 0.02	−8	0.59 ± 0.01	−8	1.16 ± 0.05	−10	2.41 ± 0.13	−6
1,3-Dichlorobenzene	0.31 ± 0.02	−4	0.60 ± 0.01	−7	1.15 ± 0.04	−10	2.22 ± 0.13	−13
1,4-Dichlorobenzene	0.35 ± 0.02	8	0.64 ± 0.01	−1	1.20 ± 0.05	−6	2.31 ± 0.11	−10
2,5-Dimethylfuran	0.29 ± 0.03	2	0.58 ± 0.01	2	1.19 ± 0.03	4	2.40 ± 0.14	5
Benzene	0.30 ± 0.03	−6	0.59 ± 0.02	−8	1.16 ± 0.04	−9	2.28 ± 0.07	−11
Bromodichloromethane	0.32 ± 0.02	0	0.64 ± 0.02	0	1.21 ± 0.05	−6	2.38 ± 0.12	−7
Bromoform	0.35 ± 0.02	8	0.68 ± 0.02	6	1.27 ± 0.03	−1	2.53 ± 0.09	−1
Carbon tetrachloride	0.26 ± 0.03	−18	0.50 ± 0.03	−22	1.00 ± 0.03	−22	2.09 ± 0.08	−18
Chlorobenzene	0.29 ± 0.02	−8	0.57 ± 0.02	−10	1.12 ± 0.03	−12	2.23 ± 0.11	−13
Chloroform	0.31 ± 0.03	−4	0.60 ± 0.02	−6	1.18 ± 0.03	−8	2.31 ± 0.12	−10
cis-1,2-Dichloroethene	0.32 ± 0.02	0	0.63 ± 0.01	−2	1.16 ± 0.05	−9	2.23 ± 0.10	−13
Dibromochloromethane	0.33 ± 0.02	2	0.64 ± 0.01	1	1.22 ± 0.03	−5	2.40 ± 0.10	−6
Dibromomethane	0.31 ± 0.01	−4	0.60 ± 0.02	−6	1.15 ± 0.03	−10	2.33 ± 0.10	−9
Ethylbenzene	0.29 ± 0.03	−10	0.57 ± 0.02	−11	1.13 ± 0.01	−12	2.28 ± 0.11	−11
Hexachloroethane	0.29 ± 0.03	−10	0.57 ± 0.02	−10	1.15 ± 0.03	−10	2.23 ± 0.10	−13
m/*p*-Xylene	0.63 ± 0.05	−2	1.25 ± 0.04	−2	2.4 ± 0.05	−6	4.50 ± 0.22	−12
Methylene Chloride	0.28 ± 0.01	14	0.51 ± 0.05	20	1.01 ± 0.02	21	2.01 ± 0.12	22
o-Xylene	0.30 ± 0.03	−7	0.63 ± 0.02	−2	1.31 ± 0.03	2	2.98 ± 0.19	17
Styrene	0.29 ± 0.02	−10	0.56 ± 0.01	−12	1.13 ± 0.03	−11	2.33 ± 0.13	−9
Methyl *tert*-butyl ether	0.32 ± 0.02	−1	0.60 ± 0.01	−6	1.19 ± 0.02	−7	2.22 ± 0.13	−13
Tetrachloroethene	0.41 ± 0.04	4	0.83 ± 0.03	6	1.56 ± 0.01	0	3.52 ± 0.25	13
Toluene	0.29 ± 0.02	−10	0.56 ± 0.02	−12	1.11 ± 0.02	−13	2.23 ± 0.10	−13
trans-1,2-Dichloroethene	0.31 ± 0.04	−2	0.60 ± 0.02	−7	1.19 ± 0.05	−7	2.31 ± 0.04	−10
Trichloroethene	0.35 ± 0.03	9	0.67 ± 0.01	4	1.34 ± 0.03	5	2.53 ± 0.14	−1

[a] Proficiency testing.
[b] Standard deviation.
[c] % Difference.

analysis of these aqueous proficiency testing samples over a 12-month period produced the results shown in Table 4. Each of the four proficiency testing pools was analyzed six times, and the resulting means and standard deviations were tabulated for each analyte. If the difference between the measured and true levels divided by true level exceeded ±25% for more than one of the concentrations for an analyte, then that analyte failed the proficiency testing. No analytes exceeded the 25% threshold (Table 4). Proficiency testing revealed consistent bias for some analytes, such as 1,1-dichloroethene, with measured results falling 17–25% lower than expected. This bias may have resulted from diffusion loss during preparation of the proficiency testing material. Methylene chloride also was problematic during proficiency testing. An adjacent laboratory used methylene chloride in liter quantities daily as an extraction solvent, probably leading to the laboratory air contamination that resulted in variable shifts in our data for that analyte. Analytical blanks were used to estimate this contamination and to ensure that analyses were not unduly affected. This variable contamination led to definition of the lowest reportable value for methylene chloride at a much higher level (0.07 μg/L) than the limits defined by absolute instrument response (0.017 μg/L).

We examined the stability of the VOC analytes by fortifying whole blood with a known amount of each analyte and storing the samples in specially treated Vacutainers for varying amounts of time (Table 5). VOC levels did not change substantially when samples were stored during a 10-week period at 4 °C. Most analytes showed only a modest drop in levels (6% average decrease) on storage for 10 weeks, possibly indicating some migration of analyte into the butyl rubber stopper. Samples began to putrefy with time and became increasingly more difficult to handle after 10 weeks of refrigerated storage. On the basis of these results, we attempt to analyze all samples within 2 weeks of collection but no later than 10 weeks after collection.

This method offers significant improvements over previously published VOC methods [14,16] by allowing us improved safety and throughput for a large number of VOC analytes. Analysis of purge-and-trap samples requires sparging human blood, a labor-intensive process that is not amenable to high throughput. By contrast, SPME allows automation of more samples, increasing throughput and thus allowing for conduct of larger studies. Purge-and-trap extraction is more efficient than SPME at extracting volatiles from a blood sample. Previous studies estimate absolute extraction efficiencies to range from 10 to 20% [14] compared with 5% to 15% absolute recoveries when using our SPME headspace extraction method. The use of nonequilibrium SPME parameters led to incomplete extraction of volatile analytes in the sample. The limited absolute recovery of analytes during SPME extraction was particularly poor for *tert*-butyl alcohol. Therefore, this volatile alcohol was not included in the SPME–GC–MS method. Limited absolute recovery of analytes had the advantage of allowing for multiple extractions of a prepared blood sample, although samples must be reanalyzed within several hours of initial extraction to minimize contamination of the sample from laboratory air. Absolute recovery of analytes decreased with each SPME headspace extraction of a sample; however, the quantified amount remained constant because of proportional loss of the stable isotope-labeled analog. Absolute extraction efficiencies were consistent with those predicted by partitioning coefficients [25]. For example, chloroform was extracted more efficiently than was MTBE or 1,4-dichlorobenzene.

Table 5
Stability of VOC analytes in stored human blood samples (μg/L)

Analyte	Time zero	10 week storage
1,1,1-Trichloroethane	0.118 ± 0.035	0.116 ± 0.039
1,1,2,2-Tetrachloroethane	0.061 ± 0.014	0.054 ± 0.005
1,1,2-Trichloroethane	0.081 ± 0.012	0.075 ± 0.005
1,1-Dichloroethane	0.038 ± 0.008	0.040 ± 0.013
1,1-Dichloroethylene	0.045 ± 0.004	0.049 ± 0.022
1,2-Dichlorobenzene	0.076 ± 0.004	0.080 ± 0.006
1,2-Dichloroethane	0.054 ± 0.011	0.052 ± 0.011
1,2-Dichloropropane	0.065 ± 0.009	0.067 ± 0.021
1,3-Dichlorobenzene	0.068 ± 0.022	0.058 ± 0.015
1,4-Dichlorobenzene	0.537 ± 0.169	0.527 ± 0.089
2,5-Dimethylfuran	0.093 ± 0.016	0.095 ± 0.009
Benzene	0.144 ± 0.019	0.126 ± 0.010
Bromodichloromethane	0.136 ± 0.024	0.121 ± 0.010
Bromoform	0.232 ± 0.050	0.217 ± 0.010
Carbon tetrachloride	0.020 ± 0.010	0.018 ± 0.009
Chlorobenzene	0.045 ± 0.010	0.041 ± 0.012
Chloroform	0.144 ± 0.041	0.131 ± 0.023
cis-1,2-Dichloroethene	0.076 ± 0.002	0.076 ± 0.005
Dibromochloromethane	0.053 ± 0.011	0.048 ± 0.002
Dibromomethane	0.040 ± 0.002	0.031 ± 0.006
Ethylbenzene	0.126 ± 0.028	0.100 ± 0.017
Hexachloroethane	0.059 ± 0.018	0.047 ± 0.006
m/*p*-Xylene	0.294 ± 0.084	0.282 ± 0.066
Methylene chloride	0.145 ± 0.019	0.117 ± 0.009
Methyl tert-butyl ether	0.238 ± 0.018	0.220 ± 0.025
o-Xylene	0.075 ± 0.021	0.070 ± 0.015
Styrene	0.274 ± 0.075	0.246 ± 0.150
Tetrachloroethene	0.187 ± 0.044	0.184 ± 0.074
Toluene	0.162 ± 0.035	0.167 ± 0.042
trans-1,2-Dichloroethene	0.081 ± 0.025	0.082 ± 0.033
Trichloroethene	0.046 ± 0.009	0.047 ± 0.005

During a 2-year period, we used this SPME–GC–MS method to analyze 951 blood samples from a geographically diverse American population. In most of these samples, the method quantified detectable blood levels of benzene, 1,4-dichlorobenzene, ethylbenzene, toluene and *m*/*p*-xylene. Some of the blood samples also contained detectable levels of 13 additional analytes: styrene, chloroform, *o*-xylene, tetrachloroethene, 2,5-dimethylfuran, MTBE, bromodichloromethane, dibromochloromethane, bromoform, carbon tetrachloride, trichloroethene, methylene chloride and 1,2-dichloroethane. Further steps to decrease contamination and improve sensitivity may improve LODs and increase the percentage of the population for which we can characterize exposure to these analytes. These detection frequencies are similar to those previously found in the general US population using purge-and-trap coupled with high-resolution MS [1], yet with higher throughput, improved ruggedness, and reduced cost. This improved method will help elucidate the human health relevance of environmental VOC exposure.

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