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Analysis of human breath samples with a multi-bed sorption trap and comprehensive two-dimensional gas chromatography (GC × GC)

Mark Libardoni [a,*], P.T. Stevens [a], J. Hunter Waite [b], Richard Sacks [a]

[a] *Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, United States*
[b] *Department of Atmospheric, Oceanic and Space Science, University of Michigan, Ann Arbor, MI 48109, United States*

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Abstract

A multi-bed sorption trap designed to quantitatively collect volatile organic compounds from large-volume vapor samples and inject them into a gas chromatograph is combined with a comprehensive two-dimensional gas chromatograph (GC × GC) for the analysis of organic compounds in human breath samples. The first-column effluent of the GC × GC is modulated by a single-stage, resistively-heated and air-cooled segment of 0.18-mm i.d. stainless steel column using the same stationary phase as the first column. Cooling gas is provided by a two-stage conventional refrigeration system, and thus no consumables other than carrier gas and electric power are required. The sorption trap uses four discreet beds, three containing different grades of graphitized carbon and one containing a carbon molecular sieve. The ordering of the beds in the trap tube is from the weakest to strongest adsorbent during sample collection. Breath samples are collected in gas sampling bags, and samples are passed through the trap at a flow rate of about 50 cm^3/min. After sample collection, hydrogen carrier gas flow is initiated in the direction opposite to the sample collection flow, and the metal trap tube is resistively heated to inject a sample plug into the GC × GC. Performance data for the combined GC × GC/sorption-trap instrument is described, and human breath-sample chromatograms are presented.

Keywords: GC × GC; Human breath analysis; Instrument design; FID

1. Introduction

The analysis of human breath samples has great potential as a minimally invasive medical diagnostic method as well as a means for monitoring human exposure to environmental toxins. Volatile organic compounds (VOCs) in human breath were identified as early as 1970 [1]. Emission of VOCs from human breath includes hydrocarbons, alcohols, ketones and aldehydes at ppt to ppm levels. Equilibrium in the lungs between VOCs dissolved in blood and the lung gases provides the opportunity for the monitoring of these compounds in the gas phase, rather than in the liquid (blood or urine) phase. In the lungs, only a thin barrier separates the air in the alveoli from the blood in the capillaries [2]. This barrier is called the pulmonary alveolar membrane. The underlying mechanism for breath analysis is the relatively rapid equilibration between alveolar air and pulmonary blood. This is based on partitioning into the membrane and passive diffusion across it [3,4]. Therefore, analysis of VOCs in breath should be an excellent indicator of the levels of these components in blood.

Interest in analyzing human breath for VOCs is a result of the identification and correlation of certain components with a variety of diseases [5,6]. For example, volatile sulfur compounds are related to hepatic diseases and malodor [7]. The presence of straight-chain hydrocarbons is a result of lipid peroxidation of polyunsaturated fatty acids found in cellular membranes [8]. Increased levels of hydrocarbons have been associated with pulmonary, liver, autoimmune, bowel and neurological diseases [3,9,10]. Other VOCs have been identified as markers of more specific pathologies such as isoprene for hypercholesterolemia and acetone for diabetes [11–13]. In addition, researchers are investigating certain markers related to cancer, transplanted organ rejection and trace level contaminants leeched into the blood from dialysis tubing [14–19].

Analysis of human breath samples by GC and GC–MS is complicated by the very low concentrations of many organic

* Correspondence to: 815 Pilot Rd. Suite C, Las Vegas, NV 89119, United States. Tel.: +1 702 614 1143x227.
E-mail address: mark_libardoni@leco.com (M. Libardoni).

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compounds and the large number of compounds that have been detected [20]. Several hundred organic compounds have been detected in breath samples, and in one case involving breath samples from 50 nominally healthy subjects, more than 3400 different VOCs were observed [21]. Healthy individuals differ widely in the composition of their breath with fewer than 30 shared compounds found in the breath of all humans. Major VOCs present in human breath include isoprene, acetone, ethanol, methanol and other alcohols. Minor components include pentane and higher aldehydes and ketones.

Because of the very low concentrations of many compounds in breath, some preconcentration is required prior to analysis. Sorption-based traps have been used for the preconcentration of organic compounds from large-volume vapor samples, but thermal desorption often results in very wide injection plugs, and a cryogenic-focusing device may be needed between the sorption trap and the GC in order to obtain sufficiently narrow injection plugs [22–25]. Another drawback of sorption traps is the potential for thermal degradation of labile compounds during desorption. This has been shown to be a significant problem for some biogenic compounds including aldehydes and terpenes [26,27].

Membrane extraction with a micro-scale trap interface has been used for the collection and injection of organic compounds from large-volume samples with single dimension GC analysis [2]. The hydrophobic membrane excludes water vapor from the trap, but may also reduce recovery for some sample components. Similarly, a multi-bed sorption trap has been designed and evaluated in our laboratory [28]. This design has been used for the direct collection and injection of organic compounds from large-volume breath samples into a single-dimension GC [29]. Experimental results determined that water vapor is not strongly retained on the carbon-based sorbents. With hydrogen carrier gas, biogenic compounds such as α- and β-pinene can be quantitatively desorbed from the trap at 300 °C with minimal decomposition.

The very large peak capacity and high sensitivity of comprehensive two-dimensional GC (GC × GC) make it an excellent candidate for the analysis of organic compounds in human breath [30,31]. Comprehensive two-dimensional separations are achieved by connecting in series two capillary columns using different stationary phases by means of a concentration-modulator interface. For thermal modulators, the device is cooled to collect sequential portions of the first column effluent and periodically heated to inject a series of narrow plugs for fast separation on the second column. High peak capacity is achieved from chromatograms defined on a two-dimensional retention time plane rather than on a single retention-time axis. High sensitivity is achieved by the use of an efficient modulator, which injects very narrow vapor plugs into a micro-bore column for a high-speed, second-column separation. Another attractive feature of GC × GC is that more structured chromatograms are obtained, and the position of a peak in the retention plane provides information useful for the classification of compounds found in breath samples.

Since its initial development by John Phillips in the late 1980s, the thermal modulator has taken on different designs to provide the required heating and cooling [32]. Thermal modulators usually are mass conserving and can provide greater detection enhancement than devices using valves and sample loops. However, the latter devices require no cryogenic materials. Both thermal and pneumatic modulators have been described in several recent reviews [33–35].

Recent work in our laboratory described the design and performance of a single-stage, resistively-heated and air-cooled thermal modulator that uses no consumables other than carrier gas and line power. It can provide modulated peak widths at half-height of under 20 ms [36]. The work presented in this report describes a GC × GC/sorption-trap instrument which utilizes a single-stage resistively-heated and air-cooled thermal modulator for the comprehensive analysis of human breath samples. Only electrical power and carrier gas are required for instrument operation. The GC × GC is combined with the multi-bed sorption trap for qualitative and quantitative analysis of organic compounds in the volatility range from about *n*-C_5 to *n*-C_{13}.

2. Experimental

2.1. Apparatus

A diagram showing major instrument components is presented in Fig. 1. The inset shows detail of the multi-bed trap. An HP 5890 GC is used as an experimental platform. The thermal modulator and the second (high-speed) column are located in the GC oven. The HP flame ionization detector (FID) is used with a fast (200 Hz) electrometer (Chromatofast Inc., Ann Arbor, MI). Independent temperature control of the two columns is achieved by locating the first column outside the GC oven and using at-column heating [37]. The 30-m long, 0.25-mm i.d. first column uses a 0.25-μm film of dimethyl polysiloxane (Rtx-1, Restek Corp., Bellefonte, PA). The column and co-linear heater wire and sensor wire are wrapped with fiber insulation. This ensemble is wound in a coil and the coil wrapped with metal foil. The column was prepared by RVM Scientific, Santa Barbara, CA. The temperature controller was also provided by RVM Scientific. The second column, which is located inside the 5890 oven, is 1.5-m long, 0.1-mm i.d. and uses a 0.1 μm film of polyethylene glycol (Rtx-Wax, Restek Corp.).

2.2. Modulator design

The single-stage thermal modulator uses an 8.0-cm long segment of 0.18-mm i.d. fused-silica-lined stainless steel tubing with a 0.18-μm thick film of dimethyl polysiloxane (Mxt-1, Restek Corp.). The center 5.5 cm of the modulator tube is heated by way of electrical contacts made directly to the modulator. The modulator is housed in a machined aluminum block containing a 1.6-cm o.d., 1.0-cm i.d., 5.0 cm long ceramic tube. Holes in the ceramic tube provide for the cooling air flow. The holes in the ends of the aluminum block are sealed with standard 11 mm injection-port septa. The modulator tube passes through the center of each septum, which provide a gas-tight seal. The entire housing containing the modulator tube is wrapped with high

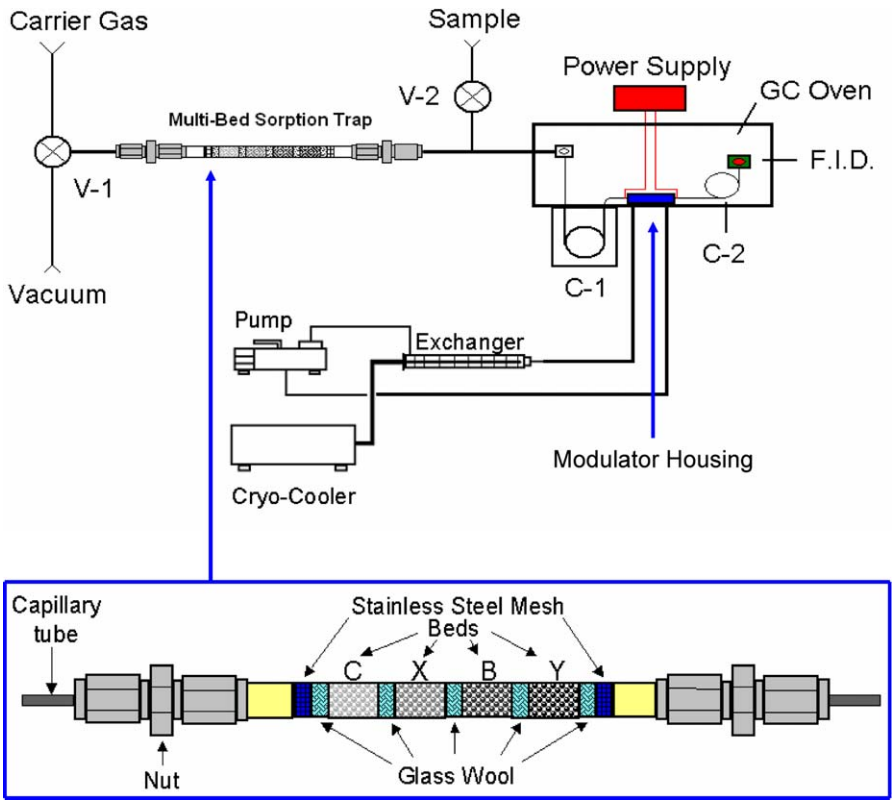

Fig. 1. Schematic of the GC × GC instrument used for human breath anaysis. A multi-bed sorption trap is used for sample collection and introduction into the GC × GC-FID. See text for sampling and analysis details.

temperature Kevlar tape and mounted to the inside wall of the GC × GC oven.

The modulator tube is resistively heated by the current from an adjustable-voltage dc power supply (Model DS-304M, Zurich MPJA, Lake Park, FL). A 0.5-s long heating pulse is applied to the modulator tube every 5.0 s. The pulse voltage and current are 4.44 V and 3.44 A (average), respectively. Heating pulse timing is controlled with a PC by means of a solid-state relay (RSDC-DC-120-000, Continental Industries Inc., Mesa, AZ).

Modulator cooling is provided by cold air from a conventional refrigeration unit (Model CC-100 Cryocool Immersion Cooler, Neslab Instruments, Portmouth, NH) by means of a heat exchanger built in house. A re-circulating pump is used to prevent ice accumulation in the heat exchanger. The cold-air flow rate was 35 L/min, and the air exiting the heat exchanger had a temperature of −45 °C. The device is very low maintenance and requires only line voltage for its operation.

2.3. Multi-bed trap design

The trap used for sample procencentration was constructed in house and has been described in detail [28]. In brief, the trap consists of a 8.0-cm long, 1.35-mm i.d. Inconel 600 (Co–Ni alloy) metal tube (Accu-Tube Corp., Englewood, CO) packed with four discreet sorption beds indicated as Y, B, X and C in Fig. 1. Three of the beds use different grades of graphitized carbon (Carbopack Y, B and X, Supelco, Bellefonte, PA), and the forth bed (C) consists of carbon molecular sieves (Carboxen 1000, Supelco). Each bed contains about 2.2 mg of sorbent. The beds are separated by plugs of glass wool, and the bed ensemble is retained in the trap tube by plugs of stainless steel mesh. The beds are ordered from weakest to strongest (largest surface area) from right to left in Fig. 1. The multi-bed trap was conditioned off-line at 250 °C for 2 h under a constant flow (75 mL/min) of dry nitrogen.

A vacuum pump (KNF, UN86 KNI, KNF-Neuberger, Trenton, NJ) is used to pull sample gas from right to left through the trap tube in Fig. 1. Sample flow rate is 50 cm^3/min. Valves V_1, a three-way valve (01380-05, Cole Parmer, Vernon Hills, IL) and V_2, a two-way valve (LFVA1230113H, Lee Co., Inestbrook, CT) are used for flow control. For sample collection, V_2 is open, and V_1 is open to the vacuum pump. After sample collection is complete, valve V_2 is closed, and valve V_1 is open to the hydrogen carrier gas. Carrier gas flows through the trap tube from left to right. During sample collection, the weakest adsorbent (bed Y) strips the least volatile components from the sample. The process continues, and only the most volatile and polar compounds are collected in the strongest adsorbent (bed C). After sample collection, the carrier gas flow through the trap is reversed relative to the direction from that of the gas flow during sampling. This prevents the least volatile compounds from ever reaching the strongest adsorbent from which they would be very difficult to thermally desorb. This results in quantitative desorption with no significant memory effects.

Thermal desorption is accomplished by resistively heating the metal trap tube to about 300 °C by means of current pulses from two ac autotransformers. Initial heating to about 300 °C is obtained from a 1.0-s long, 8.4 V pulse, and the trap temperature is maintained at this value by a 15.0-s long, 2.8 V pulse. This produces a 1–2 s wide injection plug for the GC × GC. The heating pulses are controlled by means of solid-state relays (RSDC-DC-120-000, Continental Industries, Leesburg, VA) and LabTech Notebook software (LabTech, Andover, MA). This methodology allows the multi-bed trap to remain on-line and be reused. The multi-bed trap used for this study has more than 300 injections and is currently being used for further breath analysis investigations.

2.4. Materials and procedures

The standard HP 5890 electrometer lacks a sufficiently small time constant for monitoring the very narrow peaks from the second column, and a connection was made directly from the FID collector electrode to the high-speed electrometer. This necessitated operating the FID with an open-circuit ground. In order to reduce noise, the collector electrode assembly and the wire leading to the electrometer were wrapped with a grounded metal foil sheath. Despite this, the noise level was about 10 fold greater than with the standard HP electrometer. Data from the electrometer are sampled at 100–200 Hz by means of a 16-bit A/D board (PC1-DAS1602/16, Measurement Computing, Middleboro, MA) and a PC. Data are processed by Grams Spectral Notebook software (Thermo Galactic, Salem, NH). Peak volume integration, template overlay and display were performed with MatLab software (The Math Works, Natick, MA).

Test mixtures are prepared by injecting microliter quantities of either single components or neat mixtures of reagent-grade compounds into 12-L Tedlar gas sampling bags (SKC Inc., Eighty-Four, PA), diluting with compressed dry air and equilibrating for 30 min before sampling. Table 1 lists the test compounds with their respective chemical formulas and boiling points. These compounds were chosen because they have been detected in human breath samples [19,29].

Breath samples were collected in similar 1-L Tedlar gas-sampling bags. Typically, 250–800 cm^3 of a breath sample are passed through the multi-bed trap for each experiment. All of the human breath samples were collected in the morning prior to eating lunch and at least 30 min after consuming any food or beverages. Each breath sample was obtained following a deep breath, which was held for 10 s and then exhaling slowly for 10 s prior to filling the gas sampling bag.

Hydrogen carrier gas was used after purification with filters for hydrocarbons, oxygen and water vapor. The inlet pressure was set to give a flow rate of 2.2 cm^3/min at the FID. Both columns were operated with a temperature programming rate of 3.0 °C/min following a 3.0 min isothermal interval at 22 °C (room temperature) for the first column and 30 °C for the second column. The final temperatures were 175 °C for the first column and 185 °C for the second column.

Table 1
40-component mixture based on compounds found in human breath samples

No.	Name	B.P.
1	Pentane	35–36
2	Isoprene	34
3	Acetone	56
4	Ethanol	78
5	2-Propanol	82
6	Hexane	69
7	2-Butanone	80
8	Ethylacetate	77
9	1-Propanol	97
10	2-Butanol	98
11	Benzene	80
12	Isooctane	98–99
13	Heptane	98
14	2-Pentanone	100–101
15	2,5-Dimethylfuran	93
16	1-Butanol	118
17	Toluene	111
18	Octane	125–127
19	Hexanal	131
20	Butylacetate	126
21	Ethylbenzene	136
22	*m*-Xylene	139
23	*p*-Xylene	138
24	Nonane	151
25	*o*-Xylene	143–145
26	Cumene	152–154
27	α-Pinene	155
28	β-Pinene	167
29	Decane	174
30	1,2,4-Trimethylbenzene	168
31	Benzaldehyde	178–179
32	Limonene	176
33	1,2,3-Trimethylbenzene	175–176
34	1,2-Dichlorobenzene	180
35	Undecane	196
36	3-Pentanone	102
37	1-Pentanol	136–138
38	2-Heptanone	149–150
39	Dodecane	216
40	1,3-Dichlorobenzene	172–173

3. Results and discussion

3.1. Instrument performance

Carrier gas flow velocities through the columns and the thermal modulator were computed from standard equations for gas flow through capillary tubes [38]. The average and exit velocities for the first column were 28.8 cm/s and 31.2 cm/s, respectively. The base width (4σ) of the bands eluting from the first column ranged from about 20 s for the weakly retained sample components to about 30 s for the most strongly retained components. Thus, for the 5.0 s modulation period, 4–6 second-dimension chromatograms were obtained for each first-dimension peak. The average gas velocity in the thermal modulator tube was 200 cm/s, and the average velocity in the second column was 270 cm/s. The corresponding holdup times for each column are 0.04 s and 0.57 s, respectively.

The temperature of the modulator tube during trapping varied with both cooling gas flow rate and oven temperature. At the start of a run with an oven temperature of 30 °C, the modulator reached a minimum temperature of −26 °C using a cooling-gas flow rate of 35 L/min. For these conditions, the air leaving the heat exchanger was at −45 °C. For higher cooling-gas flow rates, heat exchange was less efficient, and the trapping temperature increased. At lower flow rates heat gain during transport from the heat exchanger to the modulator increased with a corresponding increase in trapping temperature. During analysis, the modulator temperature increased nearly linearly as the oven is heated, reaching a maximum temperature of 26 °C at the end of a run (oven temperature 175 °C). Even with a temperature increase in the modulator throughout the analysis due to the GC oven temperature ramp, the temperature differential between the first column and the modulator was still substantially large. This large delta T was sufficient for trapping high-boiling compounds that elute from the first column at elevated temperatures.

3.2. Chromatograms of test mixture

Fig. 2 shows the GC × GC chromatogram of the 40-component test mixture. The horizontal axis shows retention times on the first column, and the vertical axis shows retention times on the second column. The spots are the projections of the peaks onto the retention-time plane. The numbers correspond to the compound numbers in Table 1. Peaks 4, 5, 9 and 16 are all from alcohols, and they show substantially more broadening than the other compounds. This is typical of alcohols on wax columns with conventional GC as well as with GC × GC. The insets in Fig. 2 show selected portions of the chromatogram on an expanded time scale for component pairs that are only partially separated in the chromatogram. Note that component pairs 22 and 23 (*m*-xylene and *p*-xylene) and 25 and 38 (*o*-xylene and 2-heptanone) are not separated on either column and appear as single peaks in the chromatogram. The peak capacity for the chromatogram is estimated at about 1500 peaks for a resolution of 1.5.

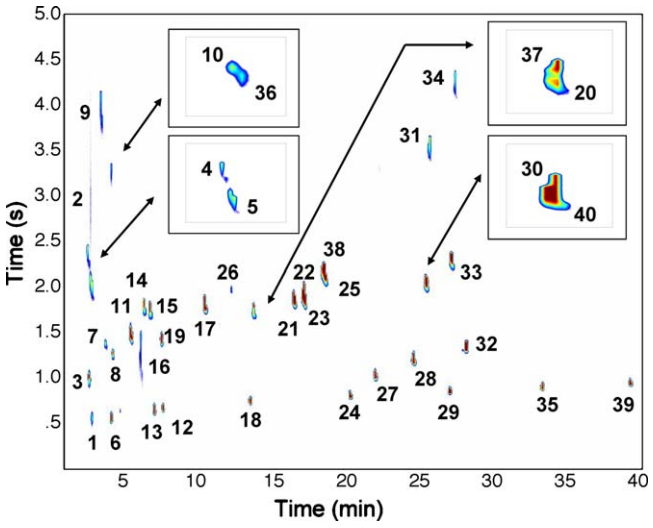

Fig. 2. Two-dimensional (contour) chromatogram of a 40-component test mixture containing compounds found in human breath. The insets show expanded views of portions of the chromatogram containing overlapping peaks. Peak numbers correspond to compound numbers in Table 1.

3.3. Quantitative analysis

The quantity of material injected into the GC can be varied by varying the concentrations in the gas-sampling bag and by varying the sampling duration. For quantitative analysis, the sums of the modulated peak areas from the linear chromatogram for a specified mixture component were computed from standards. Calibration data were collected for 13 of the compounds listed in Table 1. Calibration ranges (low ppb to low ppm) were chosen to cover approximately three orders of magnitude. Five replicate experiments were conducted for each concentration, and average peak area values were computed. Table 2 gives the mass range, linear-regression correlation coefficients, average percent relative standard deviations and detection limits [(mass/concentration) and (volume/volume)] for the 13 compounds studied. Detection limits are based on extrapolation of the plots to a signal-to-noise ratio of 3.0.

Table 2
Statistical data from calibration plots of 13 compounds found in the test mixture

No.	Compound	Mass range (μg)	v/v (ppb)	R^2	RSD (%)	LOD (pg)	LOD (ppt)
8	Hexane	1–1283	7–7790	0.998	0.83	54	216
16	Heptane	1–1189	6–6953	0.991	0.81	50	198
21	Octane	1–1101	6–6269	0.996	0.79	57	228
27	Nonane	1–1023	5–5702	0.998	0.85	48	193
32	Decane	1–953	5–5222	0.998	0.75	46	185
39	Undecane	1–892	5–4822	0.997	0.81	40	161
43	Dodecane	1–841	4–4485	0.998	0.86	37	148
7	2-Propanol	2–678	13–3459	0.993	0.86	62	250
14	Benzene	2–672	11–3078	0.997	0.71	53	211
18	2,5-Dimethylfuran	2–648	9–2871	0.998	0.76	56	222
20	Toluene	2–661	9–3060	0.999	0.65	44	174
30	α-Pinene	1–412	6–1924	0.999	0.81	68	274
35	Limonene	1–400	6–1893	0.999	0.88	66	263

Correlation coefficients are all in the range of 0.993–0.999 indicating linear peak-area response to concentration over at least three decades of concentration. Detection limits are about an order of magnitude greater than values reported in other GC × GC studies. This is the result of the need to operate the HP 5890 FID with a floating ground in order to retrofit the FID to a high-speed electrometer. However, detection limits typically are an order of magnitude lower than previously reported using the same multi-bed sorption trap and the same sample size but with a conventional (one-dimensional) separation and FID detection [28]. The very substantial reduction in detection limits is the result of the very narrow injection plug widths from the electrically-heated modulator and the subsequent narrow peaks from the second column separation. Because of the very low concentrations of many human-breath components, the lower detection limits obtained by GC × GC are of great benefit.

3.4. Human breath analysis

Typically, about half of the components detected in human breath are respired at lower concentration than the ambient concentration in the inhaled air [20]. The difference in the concentrations inhaled and exhaled reflects an increased body burden for these components and is of interest to the public health community. Components that are respired at greater concentration than in the air inhaled may be the result of metabolic processes as well as from recent diet, use of hygiene products, smoking and other non-metabolic sources. Accurate measurements from human breath samples require the use of parallel sampling of breath and environmental air so that the concentration differences (alveolar gradient) can be computed [20]. The work reported here focused on the development of a reliable, quantitative screening method with lower detection limits and enhanced separation power relative to conventional GC separations, rather than to study specific applications. Thus, the alveolar gradient was not computed, and only the respired samples were collected.

Human breath samples were collected in Tedlar gas sampling bags from co-workers on this project. Considerable interest surrounds the adsorption properties of compounds on the bag walls. Studies by McGarvey and Shorten, concluded that methanol and other small molecular weight alcohols will adsorb to the bag walls and should be analyzed within 24 h of sampling to avoid discrepancies [39]. In a more recent study, Cariou et al., investigated the use of double-wall Tedlar bags to limit humidity evolution within dry air samples [40]. All breath samples collected for this study were allowed to equilibrate for 30 min and then immediately analyzed. A sampling time of 300 s at 50 cm^3/min was used unless otherwise specified. Fig. 3 shows three-dimensional views of GC × GC chromatograms for a breath sample from two individuals. The chromatograms are very different, which is consistent with previous work showing great variability in the composition of human breath [2,3,11,16]. Factors affecting these differences include medical conditions, environmental influences and overall lifestyle. Blanks run under identical conditions using purified air as a sample inside the gas sampling bags showed only a featureless base line. Blank runs were performed before and after each breath sample.

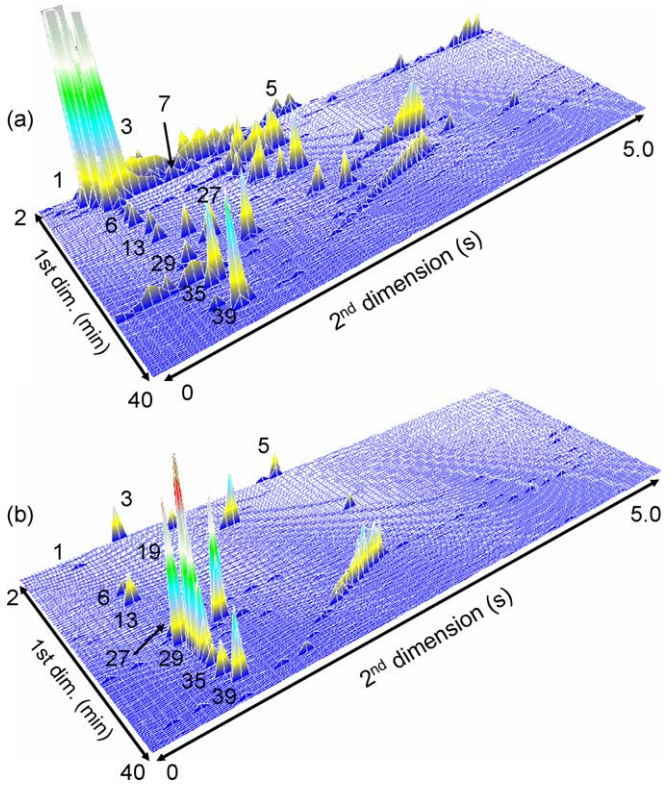

Fig. 3. Three-dimensional view of GC × GC chromatograms of human breath sample collected from two individuals. A sample collection time from the 1-L gas sampling bags of 300 s at a flow rate of 50 cm^3/min was used. Identified compounds are listed in columns 3(a) and 3(b), respectively, in Table 3.

In chromatogram 3(a), 64 peaks are observed with a signal-to-noise ratio >3.0. Two very large peaks are observed with small retention times on both columns. These peaks are for *n*-pentane (1) and acetone (3). Most of the *n*-alkanes from C_5 to C_{12} are observed with relatively large peaks for C_{11} (35) and C_{12} (39). A total of 10 compounds from the 40-component test mixture in Table 1 were detected and are listed in Table 3. These peaks were identified by means of retention time matches on both columns with the 40-component test mixture chromatogram in Fig. 2 as well as a software template overlay. The template overlay allows for visual comparison between the standards and the unknown breath samples. The numbers in Table 3, column 3(a) refer to concentrations in ppb for cases where calibration plots were obtained, and the concentration values were within the calibration range listed in Table 2. A (x) listed in Table 3 denotes an identified compound but no quantitative data. Note that some wraparound (a condition occurring when compounds on the second column do not elute prior to the next modulator cycle) is observed in chromatogram 3(a) between C_{10} (29) and C_{11} (35). This indicates the presence of very polar compounds that elute from the second column after the second modulator heating pulse occurs relative to the pulse from which the compounds were injected into the second column.

For chromatogram 3(b), 33 peaks are detected with a signal-to-noise ratio of 3.0 or greater. Acetone (3) and *n*-pentane (1), which are the largest peaks in chromatogram 3(a), are barely detectable in chromatogram 3(b). A number of higher molecular

Table 3
Compounds identified in human breath samples

No.	Name	Figure				
		3(a)	3(b)	4	5(a)	5(b)
1	Pentane	x	x	x	x	x
3	Acetone	x	x	x	x	x
6	Hexane	95	22	94	22	71
8	Ethylacetate	n/d	n/d	x	x	x
4	Ethanol	n/d	n/d	x	x	x
7	2-Butanone	x	n/d	x	n/d	x
11	Benzene	n/d	n/d	n/d	n/d	47
5	2-Propanol	137	155	135	101	411
15	2,5-Dimethylfuran	n/d	n/d	n/d	19	81
9	1-Propanol	n/d	n/d	x	x	x
10	2-Butanol	n/d	n/d	x	n/d	n/d
13	Heptane	31	7	30	1	10
12	Isooctane	n/d	n/d	x	n/d	n/d
14	2-Pentanone	n/d	n/d	x	n/d	x
17	Toluene	n/d	n/d	n/d	n/d	54
18	Octane	n/d	n/d	1	n/d	n/d
19	Hexanal	n/d	x	x	n/d	x
21	Ethylbenzene	n/d	n/d	x	n/d	x
23	*p*-Xylene	n/d	n/d	x	n/d	x
22	*m*-Xylene	n/d	n/d	x	n/d	x
25	*o*-Xylene	n/d	n/d	x	n/d	n/d
38	2-Heptanone	n/d	n/d	x	n/d	n/d
24	Nonane	n/d	n/d	1	n/d	2
26	Cumene	n/d	n/d	x	n/d	n/d
27	α-Pinene	8	6	8	8	35
28	β-Pinene	n/d	n/d	x	n/d	n/d
30	1,2,4-Trimethylbenzene	n/d	n/d	x	n/d	n/d
40	1,3-Dichlorobenzene	n/d	n/d	x	n/d	n/d
29	Decane	1	3	2	n/d	8
32	Limonene	n/d	n/d	7	x	x
31	Benzaldehyde	n/d	n/d	x	n/d	n/d
34	1,2-Dichlorobenzene	n/d	n/d	x	n/d	n/d
35	Undecane	4	21	5	1	2
39	Dodecane	11	7	12	0	1

(x) present but not quantified; (n/d) not detected.

weight alkanes from *n*-C_9 to *n*-C_{12} have relatively large peaks. The largest peak in the chromatogram is in the retention region for the xylenes and ethylbenzene. Peak area reproducibilities (RSD values) for three 250-cm^3 aliquots drawn from the same gas sampling bag are less than 1%. Identified compounds are listed in Table 3 and quantitative data are listed in column 3(b).

Fig. 4 shows the chromatogram from an 800 cm^3 (16 min sampling time) breath sample from the same individual in Fig. 3(a). Note that this is near the upper limit of human lung capacity, and larger samples will require multiple breaths. Alternatively, a lower-noise electrometer could be used with smaller samples to achieve a low limit of detection for trace components. Chromatogram 4(a) shows the two-dimensional projection (contour plot) with identified compounds. The vertical streak occurring for a first-column retention time of about 3 min is caused by severe breakthrough of acetone and *n*-pentane, which overloaded the columns and the modulator for this larger sample.

A total of 212 peaks are observed in the chromatogram at a signal-to-noise ratio of 3.0 or greater. All *n*-alkanes from C_5 to C_{12} are detected. A total of 25 compounds from the 40-component test mixture in Table 1 were detected and are listed

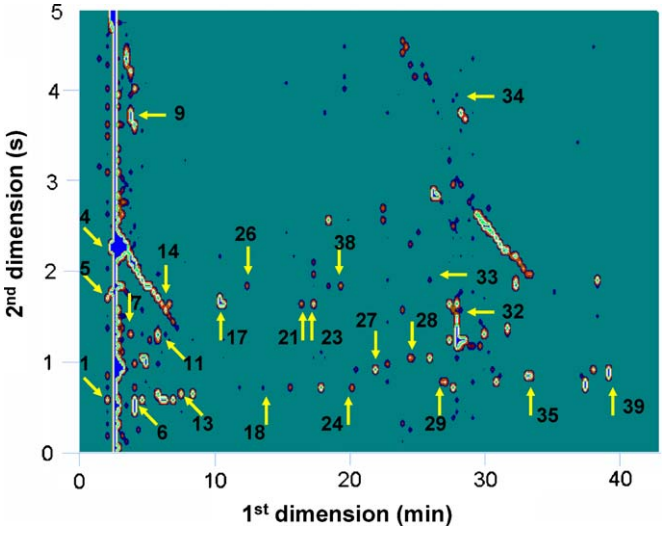

Fig. 4. Two-dimensional (contour) chromatogram of human breath sample collected form the same individual as in Fig. 3(a), but using a sample collection time from the gas sampling bag of 960 s at a flow rate of 50 cm^3/min. Identified and quantified compounds are listed in column 4(a) in Table 3.

in Table 3. Concentrations obtained by comparison with the calibration data are listed in the table for 10 of the compounds. The complexity of the chromatogram shows the usefulness of GC × GC and the available peak capacity it offers for this application.

The breath samples in Figs. 3(a) and 4 were collected about 5 min apart from the same individual in two different gas sampling bags. Note in Table 3 that the calculated concentrations for all seven components for which calibration data were available are in very good agreement indicating the overall reproducibility of the method and the efficacy of quantitative analysis of organic compounds in human breath samples at ppb levels.

Fig. 5 shows chromatograms from a smoker just before (a) and 5-min after smoking a cigarette (b). The sample size was 250 cm^3. Although an 800 cm^3 sample would have been ideal for this study, the subject was not able to completely fill a 1-L bag with one respired breath after a 10 s exhale. The latency from the previous cigarette was greater than 8 h. For chromatogram 5(a), 38 components were detected, and 11 compounds from the 40-component test mixture were identified by retention time comparison and template overlay. The number of detected peaks increases to 77 in chromatogram 5(b), and 23 components were identified from the 40-component test mixture. For visual purposes, only quantified compounds are listed in Fig. 5. All identified compounds are listed in column 5(a) and 5(b) of Table 3. Note that nearly all of the peaks present in Fig. 5(a) show increased concentration in Fig. 5(b).

Of particular interest in Fig. 5 and Table 3 is the concentration of 2,5-dimethylfuran (15) before and after smoking. This carcinogenic compound is considered a bio-marker for tobacco smoke [41]. From the calibration plot for this compound, the concentration prior to smoking was determined to be 19.4 ppb. After smoking, the concentration of 2,5-dimethylfuran increased to 81.2 ppb. This compound was not detected in any of the samples obtained from the non-smoker or the blank runs.

In another study, breath samples were collected at different times after chewing a piece of fruit-flavored gum for 5 min. Breath samples were collected at 5, 30 and 60 min after disposing of the gum. A sampling time of 300 s at 50 cm^3/min was

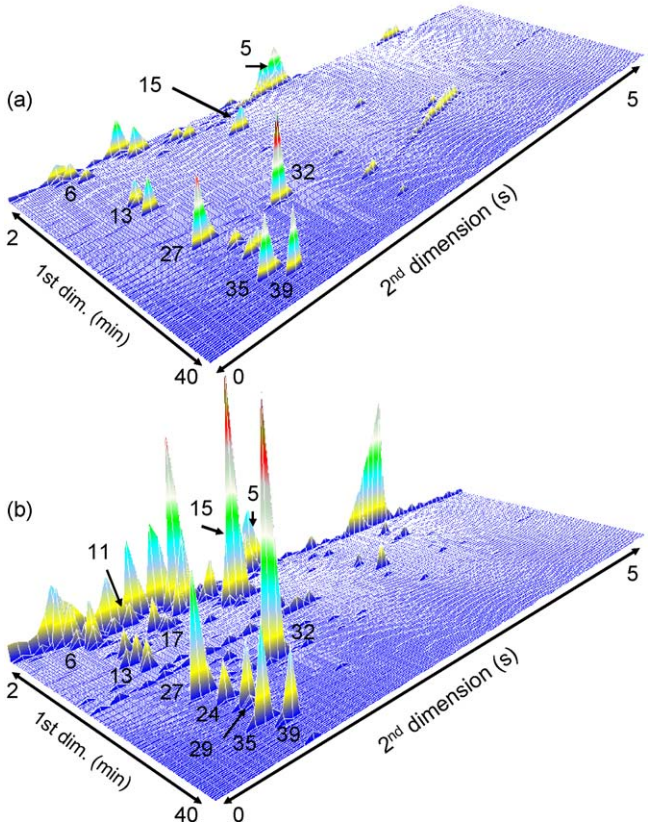

Fig. 5. Three-dimensional views of GC × GC chromatograms collected from human breath sample of an individual just prior to smoking a cigarette (a) and 5 min after smoking a cigarette (b). A sampling time of 300 s at a flow rate of 50 cm^3/min was used. Identified compounds and selected quantitative results are listed in Table 3, column 5. Peak (15) represents 2,5-dimethyl furan, a bio-marker for cigarette smoke. Prior to smoking, the concentration of 2,5-dimethyl furan was 19 ppb, 5 min after smoking a cigarette, the concentration of 2,5-dimethyl furan was 81 ppb.

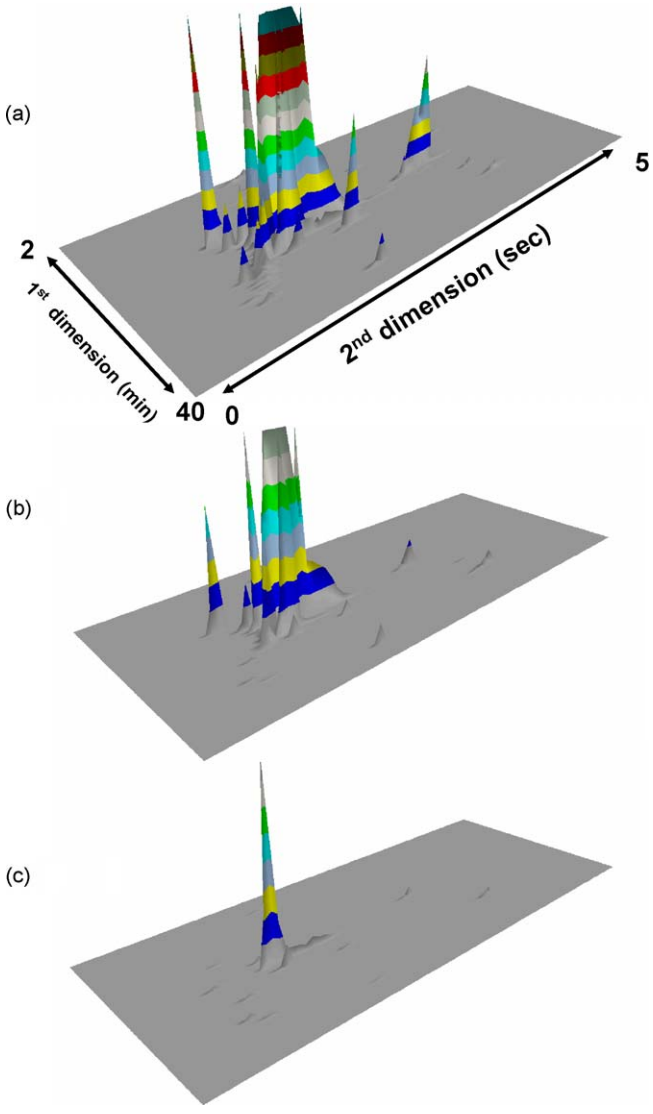

Fig. 6. Three-dimensional views of GC × GC chromatograms from human breath sample obtained from an individual 5 min (a), 30 min (b), and 60 min (c) after chewing a piece of fruit-flavored gum. Major breath components are displayed on the chromatograms and show a decrease over time.

used. Fig. 6 displays the three-dimensional views of the major components for sampling times of 5 min (a), 30 min (b) and 60 min (c). In all cases, the peak area scale has been compressed relative to previous figures so that all observed peaks are from the major breath components. The chromatograms are believed to contain peaks for mono-terpenes and other essential-oil components used for flavoring. It is clear from this figure that the elimination of these components from the body over time can be monitored.

4. Conclusions

This report has described a GC × GC system that uses a reusable multi-bed sorption trap and a single stage resistively-heated and air-cooled thermal modulator for the screening and quantitative analysis of human breath. A linear dynamic range of three orders of magnitude and detection limits in the part-per-trillion concentration range have been demonstrated and should be very useful for breath analysis. In addition to the increased detection limit, the very large peak capacity, larger than can be achieved by a one-dimensional separation in an equivalent run time, provides significantly greater specificity than conventional one-dimensional GC. Although a FID detector was used in this study, the use of a mass spectrometer would provide a greater amount of valuable information. A mass spectrometer capable of acquiring data at a fast acquisition rate would be necessary for the narrow modulated peaks that reach the detector. In this respect, a time-of-flight mass spectrometer with a fast acquisition rate would be ideal for this work. Less expensive scanning mass spectrometers are not capable of acquiring data at a suitable rate.

An important advantage of the GC × GC system described here is greatly reduced resource requirements relative to systems requiring cryogenic materials and compressed gases for modulator heating and cooling. With the present system, only carrier gas, flame gas and electric power are required. Work is in progress to further reduce the size and weight of the instrument in order to achieve portability. Current research in our lab is focused on coupling a miniaturized time-of-flight mass spectrometer to the GC × GC platform. To this end, a bread–board system with independently heated transfer lines to obviate the need for the GC platform is being evaluated. The system replaces chilled air with a liquid coolant and a relatively small liquid chiller. Preliminary work suggests enhanced performance due to the more rapid modulator cooling achieved with liquid cooling. Future work in this area will aid in the development of a portable breath analyzer for medical screening.

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