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A low cost, easy-to-assemble, open-source modular mobile sampler design for thermal desorption analysis of breath and environmental VOCs

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Abstract

Exhaled breath vapor contains hundreds of volatile organic compounds (VOCs), which are the byproducts of health and disease metabolism, and they have clinical and diagnostic potential. Simultaneous collection of breath VOCs and background environmental VOCs is important to ensure analyses eliminate exogenous compounds from clinical studies. We present a mobile sampling system to extract gaseous VOCs onto commercially available sorbent-packed thermal desorption tubes. The sampler can be connected to a number of commonly available disposable and reusable sampling bags, in the case of this study, a Tedlar bag containing a breath sample. Alternatively, the inlet can be left open to directly sample room or environmental air when obtaining a background VOC sample. The system contains a screen for the operator to input a desired sample volume. A needle valve allows the operator to control the sample flow rate, which operates with an accuracy of −1.52 ± 0.63% of the desired rate, and consistently generated that rate with 0.12 ± 0.06% error across repeated measures. A flow pump, flow sensor and microcontroller allow volumetric sampling, as opposed to timed sampling, with 0.06 ± 0.06% accuracy in the volume extracted. Four samplers were compared by sampling a standard chemical mixture, which resulted in 6.4 ± 4.7% error across all four replicate modular samplers to extract a given VOC. The samplers were deployed in a clinical setting to collect breath and background/environmental samples, including patients with active SARS-CoV-2 infections, and the device could easily move between rooms and can undergo required disinfection protocols to prevent transmission of pathogens on the case exterior. All components required for assembly are detailed and are made publicly available for non-commercial use, including the microcontroller software.

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The authors declare no competing financial interest. The software code and PCB design specifications for our sampling device are available on GitHub. Please refer to Professor Cristina Davis' webpage for more information. Commercial licensing may be available, and a license fee may be required. The Regents of the University of California own the copyrights to the software and PCB designs. Future published scientific manuscripts or reports using this software and/or hardware designs must cite this original publication (DOI:10.1088/1752-7163/ac6c9f).

We demonstrate the device collects volatile compounds, including use of chemical standards, and background and breath samples in real use conditions.

Keywords

breath analysis; exhaled breath volatiles (EBV); volatile organic compounds; thermal desorption; sampler

1. Introduction

Exhaled breath vapor (EBV) contains hundreds of endogenous volatile organic compounds (VOCs). These compounds are often the end product of metabolism, and thus reflective of health condition [1]. By measuring VOCs in exhaled breath samples, biomarker signatures of health conditions can be established, providing new clinical diagnostic tools. Researchers have demonstrated the potential for VOC-based breath diagnostics for SARS-CoV-2 infection in adults [2, 3] and children [4]; of tuberculosis [5, 6], which is distinguishable from other lower respiratory tract infections in children [7]; and to look at the absorption of inhaled, toxic polycyclic aromatic hydrocarbons (PAHs) in firefighters [8, 9].

Efforts by the breath community have been taken to standardize breath collection [10], especially with new challenges presented by the ongoing COVID-19 pandemic [11]. The breath community has begun large-scale investigations of volatile metabolites with subject populations of 700+ participants to examine potential confounding factors [12] and to quantify observed distribution of exhaled breath VOCs [13].

For studies of exhaled volatile compounds, one common method for offline chemical analysis is performed using a thermal desorption gas chromatography-mass spectrometry (GC-MS) technique. Study participants exhale directly into bags of inert plastic, such as Tedlar, in volume ranges from 0.5 to 10 L. The breath sample is loaded onto a sorbent-packed tube, which preconcentrates breath volatiles to increase detection limits. Typically, a vacuum pump is attached to the sorbent tube on one end, and the breath bag connected to the other. The pump pulls the breath sample across the sorbent material, which adsorbs or absorbs volatile compounds. These tubes can be stored, transported to a laboratory setting, and then undergo thermal desorption to release the trapped compounds, which are subsequently measured by GC-MS.

One critical step for EBV analysis is the loading of breath samples onto sorbent-packed tubes. Inconsistent tools yield inconsistent results. Within a study, varying flow rates can impact the efficiency that the sorbent material captures volatile compounds [14]. Differences in the volume of extracted breath impact the resulting VOC signal [15].

Also critical is the collection of a corresponding background air sample. Subjects inhale volatile compounds present in the room or environment around them. While the human body absorbs some, many exogenous VOCs are exhaled into the sample bag [16]. By collecting a background sample at time of breath sampling, exogenous VOCs can be subtracted from the breath sample to ensure analyses are conducted only on endogenous breath signatures.

For comparison to breath, background samples should be collected at the same time and location, using the same volume and flow rates to load background air onto thermal desorption tubes.

There are not many affordable and commercially available tools for researchers to load breath samples onto sorbent-packed thermal desorption tubes. Commercially available sampling units like the Markes ACTI-VOC (Markes International, p/n C-LFP-01) or the Gilian LFS-113 Air Sampling Pump (Sensidyne, p/n 910–0301-01) are available but are often prohibitively expensive for researchers and lack a volumetric sampling mode, rather, they use a timed-based approach where volume sampled is estimated by a function of flow rate and time. Ideally, VOC samplers would be more accessible for clinical breath researchers, especially those with multiple recruitment sites, where one sampling system cannot be shared due to geographical limitations. This way, samples can be loaded onto thermal desorption tubes at each location and returned to a “core” laboratory for analysis. We report a custom, low cost and easy to assemble VOC sampler for studies of breath vapor and environmental VOCs. The sampler contains a sample pump, flow meter and microcontroller so that breath samples are loaded volumetrically onto sorbent tubes. A display allows the operator to choose their desired sample volume, and a needle valve allows for adjustment of sample flow rate. The device is small, lightweight, and easily carried in clinical and commercial settings, allowing for collection of breath and background samples at a price under $500 US dollars in materials at the time of publishing.

2. Materials and methods

2.1. Design of VOC sampler

The VOC sampler was designed in two separate mechanical parts: a volume and flow sensing computer-controlled base station, and a modular quick changing thermal desorption tube holder. The modularity of the design will allow for future work with different sampling media using the same volume sampling hardware. The base station houses electronics, UNMP 09 diaphragm pump (KNF, Trenton, New Jersey), pressed frit filter (Vici Valco, Houston, Texas), flow sensing and control hardware, and a user interface. Additionally, a custom machined thermal desorption tube interface allows for easily and rapidly processing samples. Before first use all components in contact with breath sample were solvent cleaned with DI water, Acetone, Methanol, and isopropyl alcohol before undergoing a 24 h bake out in vacuum at 105 °C to remove any contaminations from the fabrication process. We show each of these components and their arrangement within the device (Figure 1).

Flow through the device is motivated by the KNF diaphragm pump. It pulls a vacuum through the back of the device flowing from the sample bag over the sample media and out through a filtered exhaust. An SMC AS1002F-01A (SMC, Noblesville, Indiana) needle valve is used in between the Zephyr HAFBLF0750C4AX5 flow sensor (Honeywell, Wabash, Indiana) and the pump to adjust and set flow. Additionally, this needle valve acts as a restriction to block out pressure oscillations caused by the pump that may lead to incorrect flow rate measurements.

A two-layer printed circuit board shown (Figure 2) was designed and fabricated to integrate the ESP32WROOM (Espressif Systems, Shanghai, China) wireless enabled microcontroller with a user interface, IRLB8721PbF MOSFET pump driver (International Rectifier, El Segundo , California) and Zephyr flow sensor. Each digital component, including the user interface display and flow sensor communicate over and I^2C bus, while analog switching to the MOSFET of the pump is handled by the ESP32WROOM's built-in I/O pins. A full schematic diagram of the circuit can be found in the supplemental material.

Microcontroller code included in the supplemental material, was written for the ESP32-WROOM in C++ using the Arduino developer environment. During sampling, the code uses trapezoidal integration to sum the total volume sampled by multiplying the average of the last instantaneous flow rate measurement and the current instantaneous flow rate measurement by the elapsed time of one loop of the program. After updating the counter for total volume sampled, the program then displays the instantaneous flow rate and total volume sampled to the display screen seen (Figure 3), informing the user of these parameters.

2.2. Volumetric and flow rate testing

For both volumetric and flow rate benchmarking of the device a mass flow controller, or MFC (Apex, model AX-MC-1SLPM-D/5M), was used for calibration. The MFC feedback controller was disabled, and the control valve was set to wide open to operate the MFC in a purely flow rate sensing capacity. With PTFE tubing, the MFC was connected to the sample inlet of the device.

The flow rate was assessed by comparing the flow rate as measured by the sampler device to the flow rate as validated by an external MFC The pump on the device was enabled to sample for 13 s, including an initial 3 s startup period where the pump was allowed ample time to stabilize. A time series dataset for flow rate was collected over a USB connection at 10 Hz from the device and compared to readings from the MFC recorded once every second. Both series of data from the MFC and device were averaged over the 10 s period. This experiment was performed for flowrate setpoints ranging from 100 mL/min to 500 mL/min in replicates of n=5.

Volumetric data was collected by first setting the device's needle valve and verifying the flow rate with the MFC. The breath sampler was set to sample 400 mL of volume and collection was started. Over USB connection the total sampled volume and a timestamp of each volume data point was recorded with a computer at a sample rate of 10 Hz. To analyze the volumetric measurement consistency at different flow rates, the time series data of volume versus time was plotted. The slope of this data series was representative of the average flow rate over an entire sample period. This flow rate was then compared to the flow rate set point and the one displayed on the mass flow controller.

2.3 Chemical sampling

To compare adsorption of VOCs onto sorbent tubes, a TO-15 mixture was used (Restek p/n 34434), which contained 25 gaseous VOCs at 1 ppm in nitrogen balance. Three Tedlar bags, each 5 L in volume, were filled with the TO-15 mixture. One sampler (four total) extracted

one sample from each of the three bags onto separate Tenax TA sorbent tubes (Gerstel Inc. p/n 020810–005-00), for a total of n=12 samples, which underwent thermal desorption GC-MS analysis, described below.

The twenty-five components of the TO-15 mixture are: acetone, allyl chloride, benzyl chloride, bromodichloromethane, bromoform, 1,3-butadiene, 2-butanone, carbon disulfide, cyclohexane, dibromochloromethane, trans-1,2-dichloroethene, 1,4-dioxane, ethyl acetate, 4-ethyltoluene, heptane, hexane, 2-hexanone, 4-methyl-2-pentanone, methyl tert-butyl ether, 2-propanol, propylene, tetrahydrofuran, 2,2,4-trimethylpentane, vinyl acetate and vinyl bromide.

2.4 Breath and environmental sampling

Breath sampling occurred under a protocol previously approved for human subjects research (UCD IRB #1636182). Subjects were recruited from the UC Davis and the UC Davis Medical Center. Participants were asked to not eat or drink 1 h prior to breath sampling. After consenting, subjects were coached to fill a 5 L Tedlar bag with exhaled breath using normal, tidal breathing. Immediately after, the contents of the bag were loaded onto thermal desorption tubes prepacked with Tenax TA sorbent using the VOC sampler previously calibrated to a flow rate of 250 mL/min. Samples of the background room or environment VOCs were collected at the same time and place as breath sampling. For background samples, the sampler was loaded with a sorbent tube, and allowed to extract a volume of air from the background by leaving the inlet open to the air (no Tedlar bag attached). After sampling, tubes were spiked with a first internal standard, 1 μL of 23.11 ppm chlorobenzene-d5, and stored at 4 °C until analysis. Just before analysis, a second internal standard was added, 1 μL of 500 ppb naphthalene-d8. Prior to use, sorbent tubes were conditioned by heating to 300 °C for 10 min with a flow of 50 mL/min ultra high purity helium.

At the time of publishing, over 500 breath and room samples have been collected with the device in real-world conditions, operated by doctors, nurses and clinical coordinators at the UC Davis Medical Center for the collection of breath vapor for ongoing clinical studies. The flow rate we employ in these studies (250 mL/min) was verified before each day with no observable drift. As the device was taken into patient rooms with active SARS-CoV-2 infection, the sampler was disinfected between uses by thoroughly wiping the exterior with 70% ethanol, which was not found to have carryover effects onto thermal desorption tubes.

2.5 TD-GC-MS analysis

Samples underwent TD-GC-MS analysis with a Gerstel TDU2 thermal desorption unit, CIS4 cryofocusing unit and MPS autosampler system. The thermal desorption temperature started at 30 °C and held for 3 min, then ramped 300 °C/min to a final temperature of 280 °C, holding for 5 minutes. Desorbed volatiles were led by carrier gas via a transfer line set to 280 °C to the cryofocusing CIS, which held at −100 °C. After desorption was complete, the CIS4 splitlessly injected volatiles onto the column by ramping at 12 °C/s to 280 °C, and held for 3 min. For chromatography, an Agilent 7890A GC was used, equipped with a DB-5ms column (30 m × 250 μm × 0.25 μm, Agilent Technologies Inc.). The column oven

was initially set to 38 °C for 3 min, then ramped at 3 °C/min to 110 °C, then 5 °C/min to 170 °C for 1 min, then 20 °C/min to 280 °C for 4.5 min, for a total run time of 50 min. Constant flow mode was used with 1.8 mL/min of ultra high purity helium. VOCs were detected with an Agilent 5975C single quadrupole mass spectrometer, which scanned an m/z range of 30–350, with source temperature 230 °C and quadrupole temperature 150 °C.

Alongside every batch of samples, a Grob mix was injected in triplicate to monitor instrument performance, and a Kovats mixture of C_7-C_{30} alkanes was injected to monitor retention time drift. Chemical standards were obtained from Millipore Sigma. TD-GC-MS system blanks were collected every 20 injections to ensure the system was clear of artefacts.

2.6 Data analysis

GC-MS data were deconvoluted and aligned using Profinder (version B.08, Agilent Technologies Inc.). Statistical analyses were performed using GeneSpring (Version B.14.9, Agilent Technologies Inc.), MATLAB version R2021a (The Mathworks Inc.), and PLS_Toolbox (version R9.0, Eigenvector Research Inc.). Data were $\log_{10}$ transformed, Pareto scaled and mean centered.

3. Results and discussion

3.1 VOC sampler components

Components and photographs of the VOC sampler are presented (Figure 1). This low-cost volumetric sampler can be assembled primarily with commercial off the shelf (COTS) and some easy to manufacture custom components. The device was simple to use by non-engineering clinical personnel and was implemented in a clinical setting to collect patient breath samples for lab analysis. The quick-change thermal desorption tube holder simplified operation, reducing chances of user error during a repetitive task. Furthermore, the user interface with an unambiguous display and single knob operation made the device easy for new users to learn. The device is powered by a typical wall outlet and is easily transported through laboratory and clinical spaces. In essence, the VOC sampler is a succinct and unencumbered solution for repeated sampling of VOCs for mass spectrometry by volume.

3.2 VOC sampler user interface

The front of the sampler includes a display, which allows the operator to set the desired sample volume and automatically updates the operator on sampling progress, and the progression of the screen during use is shown (Figure 3). First, the screen loads an initialization screen, which includes the software version used by the microcontroller. After initialization, the screen allows the operator to set the sample volume, using a physical knob to decrease or increase the amount, measured in milliliters. Once the volume is set and the bag and sorbent tube are loaded, the operator initiates sampling by pressing the physical knob. The screen immediately shows a “0 mL” value, which automatically updates according to the volume extracted so far. For example, if the operator sets the volume to 250 mL and initiates sampling, the display will reset to 0 mL and increase by integers [1, 2, 3, …] until the final volume is reached, in this case, 250 mL. Once complete, the screen stops at the total volume extracted while the flow system halts. To begin a new sample,

the operator simply adjusts the desired volume if needed, then initiates the next sample by pressing the physical knob.

3.3 Flow rate and volumetric accuracies

Two separate tests were conducted to verify sampler accuracy: a flow rate test followed by a volumetric test.

To verify flow rate, the sampler's needle valve was calibrated to five flow rates (100, 200, 300, 400 and 500 mL/min), with n=5 tests conducted at each rate. Summarized results are reported (Table 1 and Figure 4A). The accuracy for the sampler flow rate was slightly less than the target by an average of −1.52 ± 0.63%. This under sampling may be rooted in the calculation of volume from the sensor. Since the device calculates volume from mass flow rate, factors such as humidity, and ambient pressure cannot be automatically compensated for in the device. However, the Honeywell Zephyr sensor is automatically temperature compensated, maintaining the stated accuracy error of ± 5.5% from 0% to 95% relative humidity over the temperature range 0 to 50 °C. The inclusion of sensors to measure the previously stated conditions would improve the accuracy of the device. While the accuracy of the device is important, so is its precision. The rate that VOCs adsorb or absorb to the sorbent is impacted by flow rate. The loading of breath samples onto thermal desorption tubes is a critical step in VOC analysis, as variations in flow rates between samples within a given experiment can yield errors that confound study results. Per our results, the VOC sampler maintained the measured flow rate to within 0.12 ± 0.06% across all five tests, providing adequate precision to ensure introduction of very minimal error for loading samples onto sorbent tubes.

Results of the volumetric testing are shown (Table 2 and Figure 4B). In each of these five tests, the VOC sampler needle valve was set to one of five flow rates, from 100 to 500 mL/min. For each flow rate, n=5 experiments were conducted by setting the volume to sample as 400 mL and separately measuring the volume sampled. The largest error was a 0.61 ± 0.56 mL difference from the target volume, and overall, the error was 0.06 ± 0.06%. Likewise, the VOC sampler is highly precise. Within a given test, the five volumetric tests came within 0.05 ± 0.05% of the target volume. This ensures that sorbent tubes will be loaded with a highly accurate and precise sample volume, reducing error in experimental results.

3.4 Chemical testing of sampler

After flow rate and volumetric testing, four VOC samplers were used to extract a Tedlar bag containing a TO-15 standard VOC mixture of 25 compounds. Three total Tedlar bags were used, with each sampler collecting one sample per bag, for a total of n=12 samples. For a given VOC, the relative standard deviation for peak abundance was, on average, 6.4 ± 4.7% across three samples each from four samplers. This error rate is higher than the measured accuracy and precision of the samplers and is likely attributed to the error introduced during TD-GC-MS analysis, which can vary depending on the stability of each volatile compound through the process of thermal desorption and mass spec analysis.

3.5 GC-MS analysis of breath and background samples

Breath and environmental samples were collected onto thermal desorption tubes packed with Tenax TA sorbent using the VOC sampler to showcase its functionality in real use, clinical settings. Figure 6A shows a selected portion of raw GC-MS chromatograms, highlighting differences observed in extracting VOCs from 1 liter versus 2 liters of the same breath sample. One participant provided two breath samples, 5 L each, of exhaled breath into Tedlar bags. From each 5 L sample, the sampler was programmed to extract 1 L onto a sorbent tube, then programmed to extract 2 L onto another sorbent tube. Four samples were collected in total. In the resulting chromatograms, we see consistency from samples of equal volume as samples were collected from the same individual at the same time. Additionally, several VOCs have roughly twice the abundance in 2 L breath samples relative to 1 L. This increase in volatile abundance is expected as it correlates to the increased volume of breath samples loaded onto sorbent tubes using the extractor.

Varying the sampling flow rate affects the adsorption of volatile compounds onto sorbents. Our group previously demonstrated this on Tenax TA in a report of our custom sorbent-packed micro-preconcentrator chip [17], which has many similarities to a sorbent-packed desorption tubes herein. Here again we provide data showing this affect. A single breath sample was sampled onto sorbent tubes at three flow rates (50, 250, 500 mL/min) in 0.5 L aliquots with n=2 samples per flow rate. Figure 6B shows a selected portion of the raw GC-MS chromatograms of these results. The lowest flow rate was found to have the weakest overall VOC profile. 250 and 500 mL/min flow rates had similar VOC profiles, but relatively minor differences were observed on the abundance of certain volatiles. The difference was not always consistent; 250 mL/min provided higher sensitivity to certain compounds while 500 mL/min higher in others. Experiments targeting metabolites may need to consider the impact of flow rate to the sensitivity of their analytes of interest. For untargeted investigations of vapor, we suggest a flow rate of 250 mL/min, based on visual inspection of the overall VOC profile.

It is widely understood that a portion of compounds found in exhaled breath are exogenous, originating from the background air. Subjects may inhale these environmental VOCs and exhale them into the sample. It is critical that breath researchers collect paired environmental samples, to ensure any subsequent analyses can be void of compounds not originating from the subject. Our sampler can easily collect a background room or environmental sample by leaving the inlet open to the environment. Figure 6C shows the difference in volatile profile of a 2 L breath sample compared to 2 L of background room sample, taken at the same time and location as the breath sample. While there are instances where the same volatile compound, and even in similar abundance, appears in both breath and room samples, the chromatogram from the breath shows several VOCs unique to the breath sample, and in relatively high abundance. By using the sampler to collect these paired samples, researchers can subtract background room VOCs from the breath sample.

Figure 6D shows a latent variables plot comparing VOC profiles of n=33 breath and n=17 background, or environmental samples from multiple subjects and locations. At times, one background sample corresponded to no more than 3 subjects collected within an hour of each other at the same location. There is a distinction between the volatile profile of breath

versus background air samples. Occasionally, room samples overlap with breath profiles, indicating that those environments might have a background signal that overwhelms the breath profile, and thus these locations may not be ideal for breath analysis. This showcases the added benefit of our sampler that easily allows collection of a background sample at the same time and space as where subjects provide exhaled breath samples.

This work includes extracted breath and room samples from patients with active SARS-CoV-2 infections who were hospitalized at the UC Davis Medical Center. Between patient rooms, the device exterior was thoroughly wiped with a 70% ethanol solution, a process which was approved by the hospital clinical staff. We attempted to use commercial disinfecting wipes that are used throughout the hospital. However, these products contain volatiles such as benzyl alcohol, and they were found in very high abundances in subsequent samples (data not shown). We strongly suggest sampler operators use ethanol as a disinfectant to avoid contamination of samples with exogenous VOCs. There was no interference of ethanol with the Tenax TA sorbent used in our studies.

3.6 System background signal

There is only a minimal amount of PTFE (Teflon) tubing that the breath sample passes through during extraction onto sorbent cartridges, which connects the outlet of the Tedlar bag to the end of the sorbent-packed desorption tube. The risk of contamination from the system is thus reduced greatly. To verify the system cleanliness, we recommend researchers sample a bag of filtered nitrogen. On three occasions, a sample of filtered nitrogen was sampled after 3 consecutive breath samples. The nitrogen samples were found to be nearly void of VOCs (data not shown), indicating the system was clean and without concern of carryover, or “memory effects”, between samples. Still, periodic verification of the system cleanliness is advised.

4. Conclusion

An easy-to-assemble, open-source VOC sampler was designed for extracting VOCs from breath and environmental samples onto sorbent-packed tubes for thermal desorption GC-MS. We demonstrate that the system is highly accurate and precise, with adjustable flow rates and sample volumes. The system went through chemical standard testing and real-world breath and corresponding background samples, demonstrating its functionality in real use cases. A full description of the sampler and its components is provided.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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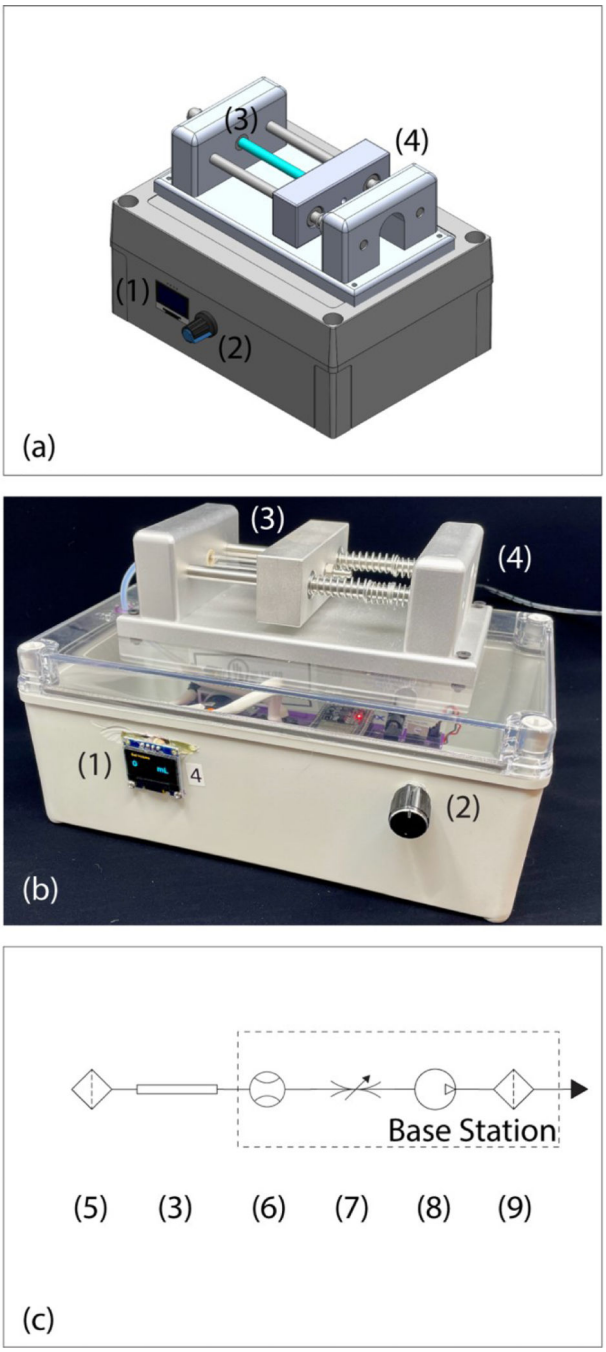

Figure 1:
The VOC sampler. (A) A CAD rendering of the metabolomic sampler including: (1) an OLED user interface, (2) rotary encoder for adjusting settings, (3) thermal desorption tube interface, and (4) spring loaded quick change mechanism for loading thermal desorption tubes. (B) a realization of the fabricated device showing the same component parts. (C) A schematic diagram of the pneumatic function of the device with (5) prefilter, (3) thermal desorption tube, (6) mass flow sensor, (7) adjustable needle valve, (8) diaphragm pump, (9) exhaust filter.

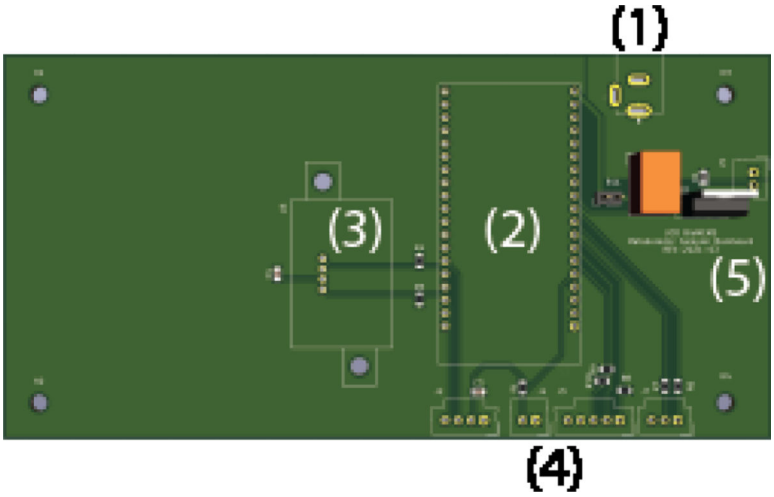

Figure 2:
Printed circuit board driving the metabolomic sampler with (1) 12 V power input, (2) ESP32 microcontroller, (3) Honeywell Zephyr mass flow sensor, (4) expansion headers for user interface, buttons and accessory pins, (5) pump driver circuitry.

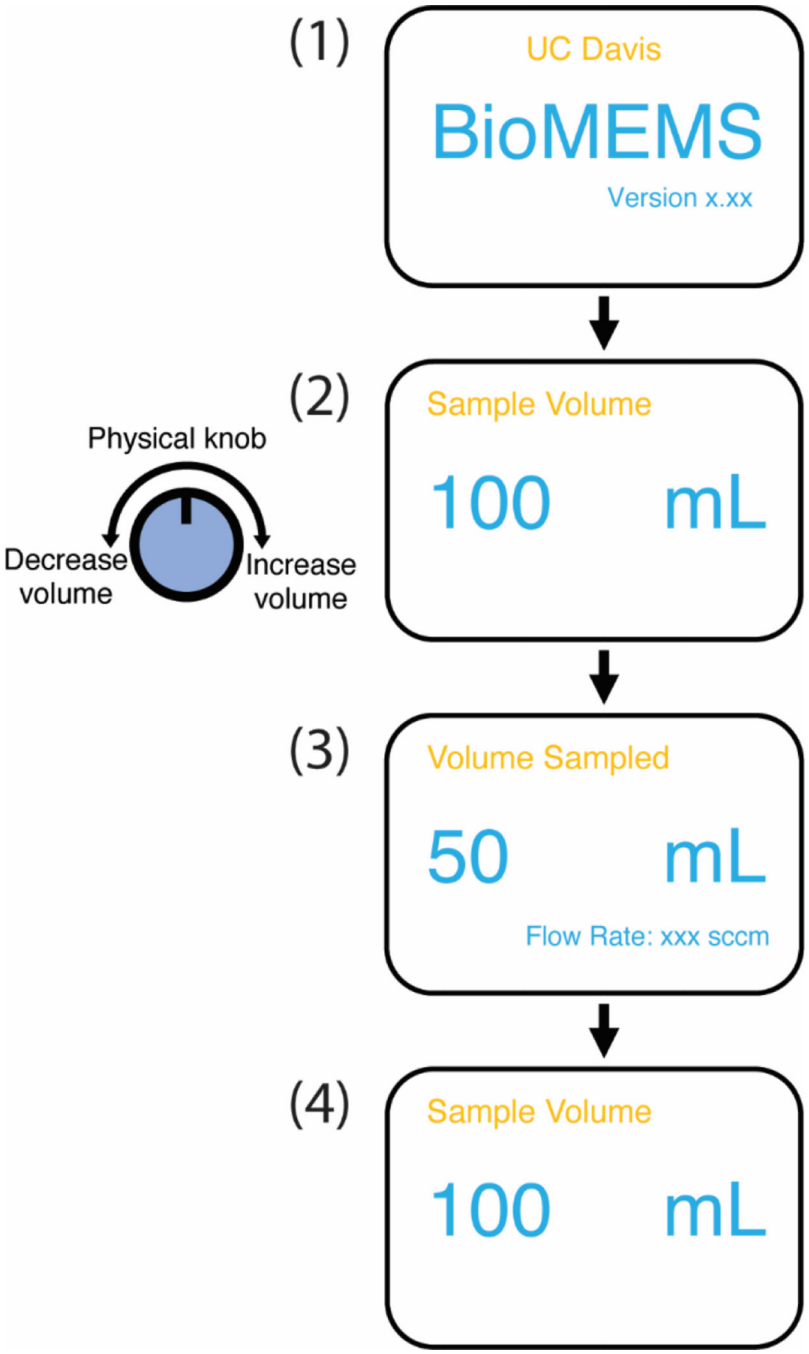

Figure 3:
Illustration of the VOC sampler display progression during sample collection. (1) Initializing screen, shown after plugging the device in. (2) Screen for the operator to adjust the sample volume, using the physical knob. (3) To initiate sampling, the operator presses the physical knob, and this screen updates with the current volume extracted, updating every second. (4) Once the final volume is reached, the screen stops at the volume collected. The operator may collect subsequent samples by replacing the thermal desorption tube and pressing the physical knob again.

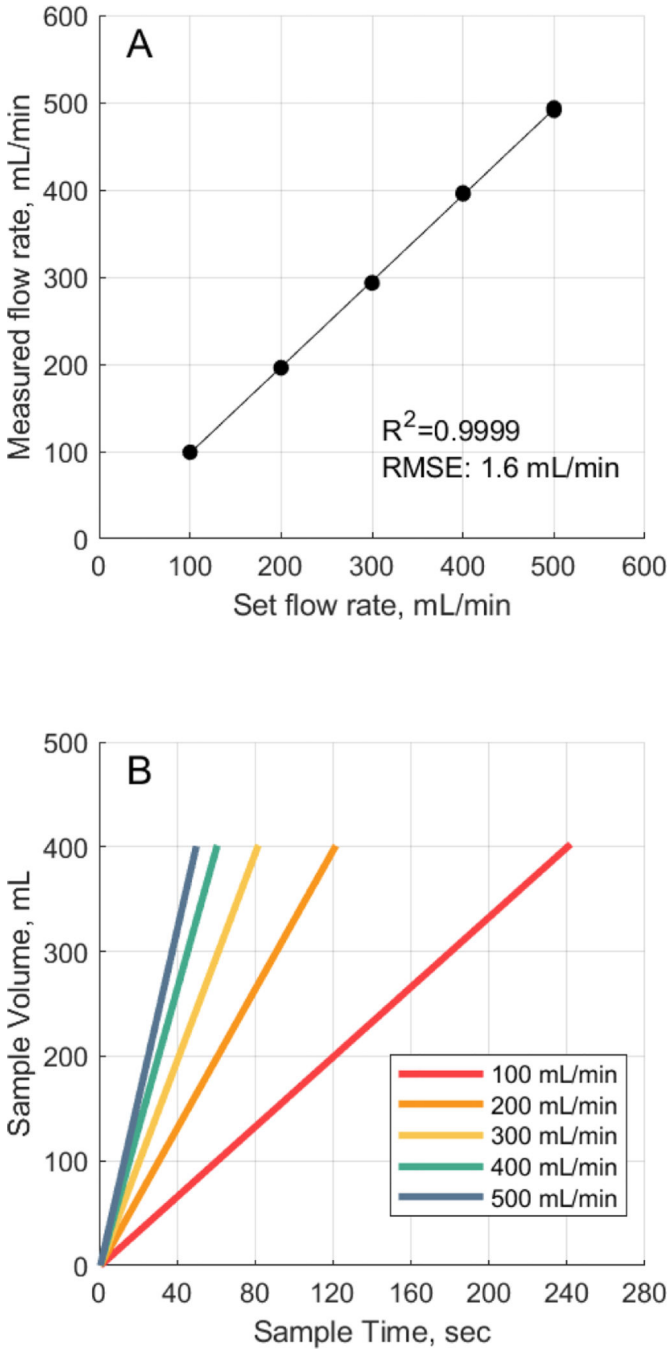

Figure 4:

(A) Flow rate testing of VOC sampler, error bars are included but are not visible, RMSE = root mean square error. (B) Volumetric testing of VOC sampler to extract 400 mL of air at 5 flow rates.

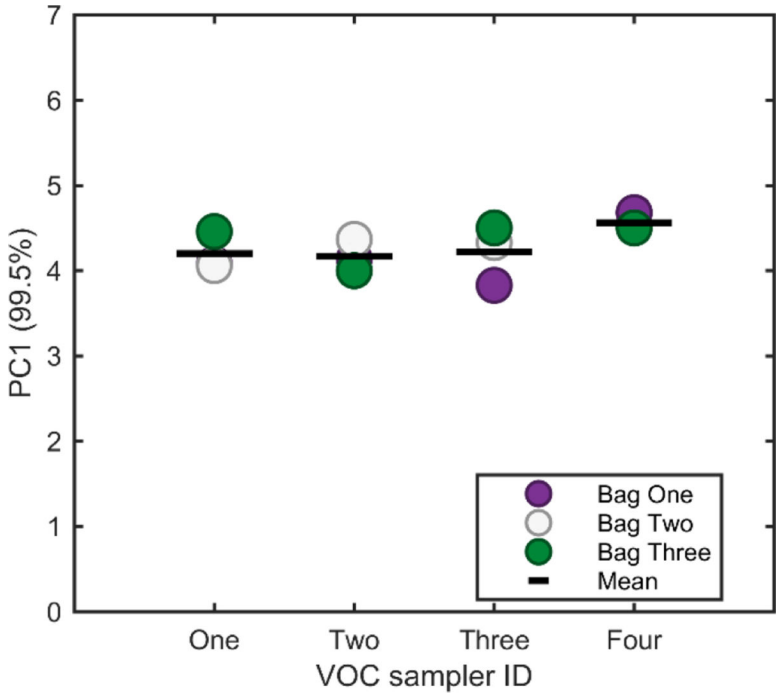

Figure 5:

Chemical standard testing of four VOC samplers. Three Tedlar bags were filled with the same TO-15 gaseous mixture of 25 VOCs. Each sampler, four total, extracted one sample from each of the 3 sample bags with the same volume and flow rate used throughout. The 1st principal component (PC1) score is shown, which is based on GC-MS data of extracted VOCs. Per ANOVA, there was no significant differences of the PC1 scores across the four extractors.

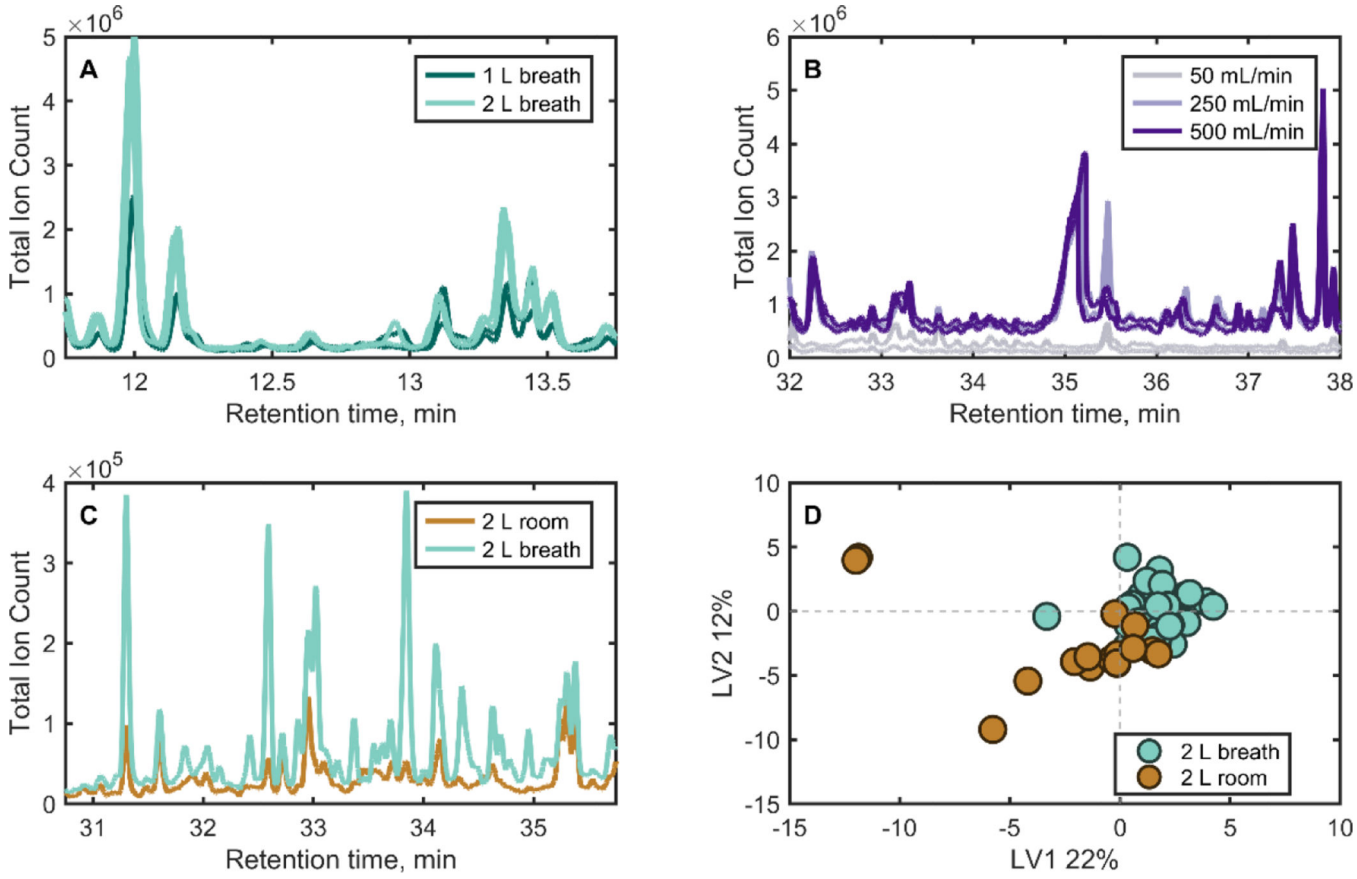

Figure 6:

TD-GC-MS chromatograms of breath and room samples collected by the VOC sampler. (A) Comparison of different volumes of the same breath sample. (B) Comparison of one breath sample onto sorbent tubes with the VOC sampler in 0.5 L aliquots, but sampled at three flow rates, showing how flow rate can impact captured VOCs. (C) Comparison of breath sample against the background air. (D) Latent variables (LV) plot comparing breath and room samples collected in clinical settings.

Table 1:

Results of VOC flow rate testing. N *=5 experiments were conducted for each of five flow rate tests. Accuracy describes how well the sampler reached the target flow rate, and precision describes how well the sampler maintained the flow rate across repeated measurements.*

	units	Test 1	Test 2	Test 3	Test 4	Test 5	Global
target flow rate	mL/min	100	200	300	400	500	
measured flow, average	mL/min	99.21	195.95	293.35	396.08	492.07	
measured flow, standard deviation	mL/min	0.09	0.14	0.30	0.50	1.09	
accuracy	%	−0.79%	−2.02%	−2.22%	−0.98%	−1.59%	−1.52 ± 0.63%
precision	%	0.09%	0.07%	0.10%	0.13%	0.22%	0.12 ± 0.06%

Table 2:

Volumetric testing of VOC sampler. N *=5 experiments were conducted for each of the five volumetric tests. Accuracy describes how well the sampler reached the target volume, and precision describes repeated attempts to reach the desired sample.*

	units	Test 1	Test 2	Test 3	Test 4	Test 5	Global
set flow rate	mL/min	100	200	300	400	500	
target volume	mL	400	400	400	400	400	400
measured volume, average	mL	399.93	400.15	400.20	400.25	400.61	400.23
measured volume, standard deviation	mL	0.06	0.14	0.15	0.11	0.56	0.20
accuracy	%	−0.02%	0.04%	0.05%	0.06%	0.15%	0.06 ± 0.06%
precision	%	0.01%	0.04%	0.04%	0.03%	0.14%	0.05 ± 0.05%