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Project Summary

Major Goals and Objectives

Our ultimate goal is to support the reliable identification of recent cannabis use by developing an infrastructure for evaluation, calibration, and quality control of cannabis breathalyzers akin to that which exists for alcohol breathalyzers. The primary psychoactive molecule in cannabis, Δ-9-tetrahydrocannabinol (THC), is semi-volatile based on challenging vapor pressure measurements made prior to the start of this project [1], and is lipophilic. The objectives of this project were, first, to identify sources of uncertainty in the capture of semi-volatile cannabinoids from breath through the investigation of one device, an impaction filter device containing replicate filters, and second, to develop robust, low-uncertainty methods to rapidly measure the volatility, or vapor pressure, of cannabis-associated compounds that might be found in breath. Human studies are used to identify cannabis-associated compounds in breath, their concentrations, and sampling challenges in the field. However, ground truth is unknown and human studies cannot be the sole means of device evaluation. Developing reference materials and delivery systems to deliver breath surrogates with known compound quantities requires relevant thermophysical properties, especially vapor pressure, to understand behavior during storage and delivery. Devices with different modes of action can be studied through human studies and numerical simulations. During this project, we utilized an impaction filter device designed to capture aerosols from breath. We examined the effect of aerosol diameter and fluid velocity on aerosol capture as a means to identify important parameters to control during human studies or when delivering breath surrogates containing aerosols. These data are essential steps towards prototyping reference materials and delivery systems for establishing ground truth for the performance of any device intended to determine recent cannabis use.

Research Questions

1. What cannabinoids are captured from exhaled breath with an impaction filter device one hour after cannabis use? How do concentrations compare with peer-reviewed studies using other devices, and what are the challenges of determining recent cannabis use from these measurements? The results of this question are published in a peer-reviewed article, Jeerage et al. [2].
2. Can vapor pressure measurements be made with low uncertainty for low-volatility and low-stability compounds that could be found in breath after cannabis use? The results of this question are published in two peer-reviewed articles, Harries et al. [3] and Beuning et al. [4].
3. How do aerosol diameter and fluid velocity affect the deposition and distribution of aerosols within an impaction filter? The results of this question are published in a peer-reviewed article, Malavé et al. [5].

Research Design, Methods, Analytical and Data Analysis Techniques for Question 1

Research Design. We conducted a pilot study that bridged the gap between highly controlled clinical studies and field studies that do not control for the time since cannabis use to investigate an impaction filter device with replicate filters [2]. Participants were recruited through a longitudinal study of cannabis use and anxiety, approved by the University of Colorado Boulder's Institutional Review Board (CU IRB No. 16-0767). Participants within the THC-dominant cannabis flower group were invited to participate in the pilot breath study, approved by NIST's Institutional Review Board (NIST IRB No. MML-2019-0182) with CU IRB relying on the NIST IRB determination through an IRB Authorization Agreement (20-0010). Participants provided two baseline breath samples on different days and one post-use breath sample approximately 1 h after cannabis use, which is within the impairment window for driving deficits identified in simulator studies [6]. Participants were instructed not to use cannabis the day before both the intake session and the experimental session. Participants were also instructed to purchase a specific THC-dominant cannabis flower product sold by a licensed dispensary to use *ad libitum* for the four weeks between the intake session and the scheduled experimental session. The experimental session was conducted within a federally-compliant mobile laboratory designed for evaluating the effects of legal-market cannabis use [7]. Baseline assessments included blood and breath samples. Then participants returned to their residence to use cannabis, *ad libitum* and unobserved by researchers (*i.e.*, naturalistic use). Once participants returned to the mobile laboratory, a blood sample was immediately collected to verify compliance with the protocol; a post-use breath sample was collected after the primary study's assessments were complete, approximately 1 h post cannabis use.

Methods. BreathExplor impaction filter devices (Figure 1) utilize eight alternating baffles in each filter to direct fluid flow and to promote capture of breath aerosols. The overall device consists of a small, injection-molded medical grade polypropylene plastic tube with a mouthpiece (Figure 1a and 1b) and three separate and parallel impaction filters (Figure 1c and 1d).

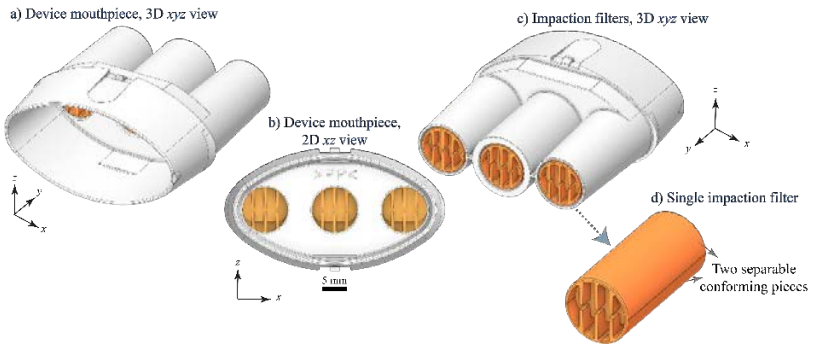

Figure 1. BreathExplor impaction filter device contains a mouthpiece (a, b) and three impaction filters in parallel (c), which can be removed for elution (d). The impaction filters are shown aligned (b, c), but are positioned randomly in real devices and are comprised of two separate pieces (d).

Participants were asked to exhale through the device following a low-lung-volume breathing maneuver. Research with non-impaired participants has shown that more aerosols are formed after full exhalation by allowing the airways to close [8,9]. The low-lung-volume breath holds have a similar, but smaller effect on aerosol production [10]. Participants provided baseline and post-use breath samples following the maneuver for 12 exhalations. More details are available in [2].

Analytical and Data Analysis Techniques. One advantage to the selected device is the three identical filters. Preliminary evidence suggests that the same breath composition results are possible [11], affording the possibility to analyze the filters separately at the roadside and in the laboratory, with one available to store for later analysis. However, the filters were analyzed together here. To prepare breath extracts, devices were warmed to ambient temperature and the filters were pushed from the housing (Figure 1a and 1b) using a disposable, manufacturer-provided tool. Each filter was submerged and soaked in 1.5 mL of methanol containing ethylene glycol. In other preconcentrating procedures, ethylene glycol has been shown to improve analyte recovery; it also provided a visual indication (pellet) of analytes for reconstitution. The combined eluent was spiked with internal standards and dried with a vacuum concentrator at 35 °C. The resulting pellet, primarily ethylene glycol containing analytes, was solvated with 100 µL of mobile phase for targeted analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS). THC identification was based on retention time (within 0.05 min) and product ion ratio (within ±20 % of its deuterated internal standard). Quantitative analysis was based on calibration standards prepared in methanol with ethylene glycol (matrix-matched) and dried and reconstituted as described above (process-matched).

Research Design, Methods, Analytical and Data Analysis Techniques for Question 2

Research Design. We developed and validated a new gas-saturation apparatus, dynamic vapor microextraction (DVME), for vapor pressure (p^{sat}) measurements on large and/or unstable compounds [3,4]. DVME was designed to be rapid, both for high throughput and to avoid decomposition of unstable compounds. The miniature equilibration vessel (the "saturator") allows for rapid measurements by decreasing thermal equilibration time. DVME was also designed to achieve state-of-the-art measurement uncertainty, to extrapolate measurements to lower or higher temperatures relevant to breath or industrial processes. Vapor pressure depends on temperature, and low-uncertainty measurements can also be used to develop an equation of state that is then used to predict values over a range of temperatures. Measurement uncertainty was minimized by reducing the total internal volume of the fluid flow path and by use of helium as the carrier gas. Helium's high thermal conductivity, low solubility, and nearly ideal mixture behavior all minimize systematic errors in the measurement. The amount of helium used for a measurement is very small (about 10,000 p^{sat} measurements can be made with a single helium cylinder), so its cost is inconsequential; however, if helium is unavailable, nitrogen gas can be used with only a modest increase

in measurement uncertainty [12]. To further facilitate adoption by other labs (this new technology is intentionally not patented), we used only commercially available components.

DVME was validated with a series of p^{sat} measurements on two compounds: the linear alkane n-eicosane ($C_{20}H_{42}$) [3] and the cannabis-associated terpene linalool ($C_{10}H_{18}O$) [4]. N-eicosane has excellent thermal and oxidative stability, it can be purchased in high purity, it has about the same molar mass as cannabinoid molecules, and it has the lowest-uncertainty p^{sat} measurements and correlations (p^{corr}) for any molecule its size [13]. Reliable p^{sat} measurements also exist for linalool [14]; however, unlike n-eicosane, linalool is prone to oxidation, is somewhat hygroscopic, and is only available at lower purities. Other terpenes will have these same challenges. Additionally, linalool's p^{sat} near room temperature is about 200 times higher than n-eicosane's, which is a challenge because of the relatively large amount of vapor produced during the measurements. Cannabinoids have about the same molar mass as n-eicosane but are expected to have lower p^{sat} values at a given temperature based on structure [1]. Additionally, most cannabinoids are prone to oxidation like linalool. There is a dearth of p^{sat} data on cannabinoids and cannabis-associated terpenes that DVME is ready to address, based on its successful validation here. DVME has recently been used to make low-uncertainty p^{sat} measurements of THC, cannabidiol (CBD), and cannabinol (CBN), with a further manuscript in preparation for submission to a peer-reviewed journal.

Methods. Designing and building the DVME apparatus (Figure 2) included selecting components based on a thorough uncertainty analysis of the measurement process and optimizing parameters for a rapid measurement that also yields low-uncertainty data. Total measurement periods as short as 15 min (3 min of thermal equilibration plus 12 min of carrier gas flow) were shown to be sufficient for high-quality p^{sat} measurements at pressures near 1 Pa [3]. The procedure for p^{sat} measurements begins by coating the glass beads in the saturator vial with the compound of interest. The saturator vial and the capillary vapor trap are installed via septa on the saturator vial and on the mass flow meter inlet and the oven is set to the desired temperature. During the thermal equilibration period a low flow of helium is used to prevent back-streaming of vapor, then the flow rate is increased for the remainder of the measurement period. At the end of the flow period, the capillary vapor trap is removed, the collected analyte is eluted with solvent into an autosampler vial containing an internal standard, and the resulting solution is analyzed by gas chromatography with flame ionization detection (GC-FID). More experimental details are available in [3] and [4].

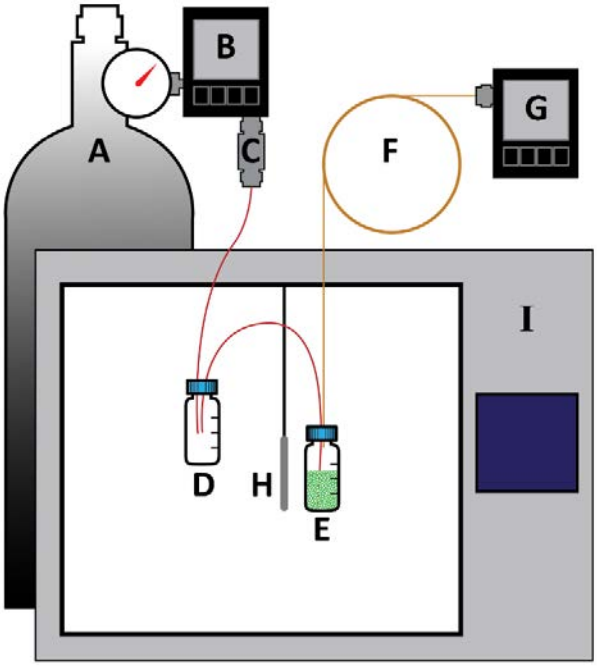

Figure 2. The primary components needed for p^{sat} measurements are a helium cylinder (A), a precision mass flow controller (B), an adsorbent tube (C), a transfer vial made from an empty 2-mL autosampler vial (D), a saturator made from a 2-mL autosampler vial containing 1-mm glass beads (E), a capillary vapor trap (F), a precision mass flow meter (G), a 100 Ω platinum resistance thermometer (H), an oven (I), and a digital barometer (not shown). The drawing is not to scale; for example, the transfer vial and saturator vial have been enlarged for clarity.

Analytical and Data Analysis Techniques. The rigorous relationship between the vapor composition and p^{sat} contains corrections for the deviation from ideal gas behavior in the vapor phase and for the Poynting correction (i.e., for the effect of applied pressure on the fugacity of the liquid phase). With helium as the carrier gas at pressures near atmospheric, these two effects are both on the order of 1% and act in opposite directions, largely cancelling each other [3]. Since the vapor nonideality correction cannot be determined in a straightforward way, we took advantage of this cancellation and used a simplified calculation with both corrections omitted,

$$p^{sat} = p \cdot y/x,$$

where p is the pressure in the saturator vial, y is the mole fraction of analyte in the vapor phase, and x is the mole fraction of analyte in the condensed phase. The value of p accounts for overpressure in the saturator vial caused by viscous flow. The value of y is determined from the total mass of carrier gas that enters the vapor trap and from the analyte mass deposited in the vapor trap as determined by GC-FID. The value of x is determined from the solubility of helium in the analyte (using Henry's law) and from the analyte purity as measured by GC-FID.

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Sources of measurement uncertainty were carefully considered (Table 1). For n-eicosane, the combined standard uncertainty in p^{sat} ranged from 2.0% to 2.8%, depending on temperature. For linalool, the combined standard uncertainty in p^{sat} ranged from 3.6% to 5.8%. The somewhat larger uncertainties for linalool are primarily due to the investigation of a lower temperature range and the use of less carrier gas, both of which compensated for linalool's higher volatility.

Source of uncertainty	**Resulting standard uncertainty in p_2^{sat}**
Mass of trapped C20 vapor (m_2)	$0.0130 \cdot p_2^{sat}$
Temperature of saturator (T)	$0.0125 \cdot p_2^{sat}$
Mass of helium carrier gas (m_1)	$0.0075 \cdot p_2^{sat}$
Simplifications in equation 1	$0.0050 \cdot p_2^{sat}$
Pressure of saturator (p)	$0.0023 \cdot p_2^{sat}$
C20 purity correction ($x_{impurities}$)	$0.0002 \cdot p_2^{sat}$
Helium solubility in C20 (x_1)	$0.0001 \cdot p_2^{sat}$
Combined standard uncertainty	$\mathbf{0.0203 \cdot p_2^{sat}}$

Table 1. Sources of uncertainty and their contribution to the uncertainty of p^{sat} measurements on n-eicosane at 344 K.

Research Design, Methods, Analytical and Data Analysis Techniques for Question 3

Research Design. We developed a high-fidelity, multiscale, three-dimensional computational fluid particle dynamics model to simulate aerosol deposition and distribution within a single filter (Figure 1d) of the device used for breath sampling (Figure 1a) [5]. Flow through the filter was simulated as two distinct phases: (a) the continuous or primary fluid phase to study velocity and kinetic energy profiles at various fluid flowrates; and (b) the discrete or secondary particle phase to trace the small, polydisperse aerosols being carried in the fluid flow. Forces and laws that govern the motion of individual particles were simplified by assuming that particles are spherical with constant diameter; particles do not rotate; particles do not undergo heat or mass transfer; and particles are dilute, so there are no particle-particle collisions. The first simulations considered the fluid phase, later simulations incorporated the particle phase, as the particles were influenced by the fluid flow, but not the reverse.

Methods. The fluid phase matched end-expiratory breath, specifically its H_2O saturation (6%) and its CO_2 concentration (5%). Aerosols were simulated as water droplets, ignoring other constituents (e.g., phospholipids and proteins). Although the density of liquid water is much larger than that of saturated air, most aerosols are expected to be 1 μm in diameter or smaller; therefore, gravitational sedimentation was neglected. We simplified reported aerosol distributions into four particle sizes (Table 2) that were "injected" simultaneously and continuously into the filter. We matched human studies by choosing the size distribution measured after a low-lung-volume breathing maneuver. Exhalation flowrates vary widely if human subjects

are not guided; for example, from 0.01 L/s to 1.2 L/s [15]. To select a lower limit, we considered the prescribed 0.05 L/s exhalation flowrate employed in the nitric oxide breath test. To select an upper limit, we considered the forced expiratory volume exhaled in 1 s, which varies by age, sex, height, and ethnicity, and ranges from 2 L to nearly 6 L. To encompass the wide range of possible exhalation flowrates, we chose 0.06 L/s, 0.6 L/s, 1.2 L/s, 2.4 L/s (plausible upper limit for females), and 3.6 L/s (plausible upper limit for males). Because the device contains three filters in parallel, we chose a constant flow of one third of the exhalation flowrate through the filter, specifically: 0.02 L/s, 0.2 L/s, 0.4 L/s, 0.8 L/s, and 1.2 L/s. Detailed descriptions of the model geometry and discretization, fluid flow dynamics governing equations, coupled fluid-particle dynamics governing equations, and the numerical solution are provided in [5].

Binned Interval (μm)	Aerosol Count (#)	Simulation Diameter (μm)	Simulation Percentage (%)
0.41-0.55	4400	0.48	51.9
0.55-0.7	2100	0.63	24.7
0.7-0.92	1320	0.81	15.6
0.92-2.98	665	1.18	7.8
TOTAL	8485		100.0

Table 2. Simplified aerosol size distribution reported by Almstrand et al. [9] with corrected diameters by Holmgren et al. [15].

Analytical and Data Analysis Techniques. Under no-slip shear flow conditions (i.e., the relative velocity between the filter surface and the fluid flow was set to zero), the particle deposition boundary condition was set so that a particle was considered deposited or "captured" upon particle-filter contact. Deposition was calculated based on the mass fraction of aerosol attached to the filter (i.e., deposition based on particle number was not registered). This allowed several aerosol fates, singly or in combination. First, *impaction*, which is the design principle behind the filter. Impaction is due to inertial forces, which occur when there is a sudden change in the direction and magnitude of the flow causing particles to deviate from flow streamlines and remain in their original path. Second, *direct interception*, due to drag forces of the flow that carry particles in the fluid streamlines and come close enough to the filter surface. Particle-filter contact is established when an edge of the particle is within one particle radius away from the filter surface, even in cases where the aerosol trajectory does not deviate from the fluid streamline. Third, *turbulent dispersion*, due to eddy forces that occur upon abrupt fluid fluctuations. Turbulent dispersion causes particles to continuously undergo motion changes due to their own non-equilibrium (unsteady) state. Fourth, *Brownian diffusion*, due to random motion of particles when interacting and colliding with fluid molecules. Finally, there

is the possibility that the particles are not captured, as no particle-filter contact occurred and the particles were carried by the fluid all the way to the filter outlet.

Expected Applicability of the Research

In the absence of reliable sensor technology that can be deployed for roadside detection, crime laboratories may be called on to extract and analyze breath samples to identify recent cannabis use. This is the approach utilized in all published studies to date: breath sampling in the clinic or field, followed by high sensitivity, high specificity laboratory analysis. While instrumental analysis and its controls eliminate some uncertainty, many uncontrolled factors remain. Furthermore, human studies, in which ground truth is unknown, cannot be the sole means of device evaluation. Our pilot-scale human study with an impaction filter device, in conjunction with peer-reviewed studies employing other filter-based devices, illustrated the challenges. One hour after cannabis use, THC collected from breath was found to vary over four orders of magnitude, with a variety of possible causes. Devices must be characterized *in vitro* and *in silico* to understand their efficiency and reproducibility, and the relative importance of human and environmental factors. Developing reference materials and delivery systems to deliver breath surrogates with known compound quantities requires relevant thermophysical properties, especially vapor pressure, to understand behavior during storage and delivery. We note that no mature breath test, clinical or forensic, relies on quantitation of semi-volatile compounds like cannabinoids [16]. Dynamic vapor microextraction (DVME), the gas-saturation method developed and validated here, yields low uncertainty vapor pressure data with high throughput, and has now been applied to THC, other cannabinoids, and terpenoids. These data are required to generate an equation of state, which can then be used to predict volatility over a range of temperatures. Numerical simulations of aerosol capture by an impaction filter were developed to identify important parameters to control during human studies or when delivering breath surrogates containing aerosols. This project has advanced the foundation for the development of reliable cannabis breathalyzer technology through identification of compounds in the breath of cannabis users as captured by an impaction filter device, development and validation of low uncertainty vapor pressure measurements for large and unstable compounds, and simulations of important human factors that affect aerosol deposition in an impaction filter. These data are essential steps towards prototyping reference materials and delivery systems for establishing ground truth for the performance of any device intended to determine recent cannabis use.

Participants and Other Collaborating Organizations

Experiments, instrumental analysis, and simulations were conducted by the Applied Chemicals and Materials Division (ACMD) within the Material Measurement Laboratory (MML) at NIST. Contributors included Dr. Cheryle N. Beuning (post-doctoral), Dr. Adam J. Friss (post-doctoral), Dr. Edward J. Garboczi, Dr. Megan E. Harries (post-doctoral), Dr. Marcia L. Huber, Bridger L. Johnston (student), Dr. Veruska D. Malavé, and Dr. Tara M. Lovestead (PI), Dr. Jason A. Widegren (PI), and Dr. Kavita M. Jeerage (PI).

NIST formed a formal partnership with Peter Stambeck, Munkplast AB and Prof. Olof Beck (retired), Karolinska University (Sweden), who provided breath sampling devices through a Material Transfer Agreement (20-034). NIST also formed a formal collaboration with Dr. L. Cinnamon Bidwell, Inst. of Cognitive Science, University of Colorado Boulder through a NIST Measurement Science and Engineering Research Grant (70NANB21H042). NIST consulted with Dr. Greg Dooley, Dept. of Environmental and Radiological Health Sciences, Colorado State University on LC-MS/MS method development.

Changes in Approach from Original Design

Our original breath sampling study would have collected breath samples from recreational users of high-potency cannabis concentrates. Due to the COVID-19 pandemic and the consequent shift to maximum telework, this study completed prior to NIST involvement. Our breath sampling study was therefore transferred to a study designed to investigate cannabis use and anxiety, meaning that our breath samples were collected from a population that may be less relevant to cannabis breathalyzer research. Additionally, we were required to modify our sampling protocol to exclude spirometry, which measures breath flowrate and volume through the breath sampling device. This is because manipulating the software to execute spirometry requires substantial interaction between (unmasked) participants and research staff in the confined space of the mobile pharmacology laboratory. This measurement would have allowed direct comparison of breath flowrate and volume to investigate sampling consistency between and within participants. Intoxication may change the ability of participants to breathe deeply through the device; this hypothesis could not be investigated. Our original proposal also focused exclusively on the behavior of vapors, proposing to measure competitive adsorption and desorption properties for a subset of cannabis compounds and breathalyzer materials with nuclear magnetic resonance (NMR) spectroscopy techniques. Despite substantial early effort, this technique did not yield acceptable uncertainties and we could not account for all material in the mass balance. Furthermore, the early results of targeted LC-MS/MS analysis of breath extracts from an impaction filter device suggested that cannabinoids are carried by aerosols, in agreement with other published studies that collected breath aerosols via filtration. Accordingly, we shifted our efforts towards understanding the behavior of sub-micron aerosols via simulations.

Outcomes

Activities and Accomplishments

- Single timepoint measurements of THC collected from breath with filter-based devices 1 h after cannabis use do not provide meaningful information about recent cannabis use.
- New DVME method is capable of vapor pressure measurements with state-of-the-art measurement uncertainty on large molecules in as little as 15 min.
- New DVME method is capable of vapor pressure measurements over a wide pressure range and with unstable molecules.
- Fluid flowrate has a dramatic effect on the deposition of aerosols in an impaction filter and both interception and impaction mechanisms are possible, depending on flowrate.

Results and Findings

Single timepoint measurements of THC collected from breath with filter-based devices 1 h after cannabis use do not provide meaningful information about recent cannabis use. Prototype cannabis breathalyzer devices have been evaluated by measuring the THC concentration in breath ($[THC]_{breath}$) at baseline and shortly after cannabis is smoked or vaped, with a known time interval (Figure 3a). While this procedure provides an indication that the prototype device is functioning as intended, it does not mimic the situation at the roadside, where no baseline measurement will be available and the time interval after cannabis use will also be unknown (Figure 3b). Furthermore, the time interval since cannabis use is likely to be 1 h or more based on perceived driving ability among recreational cannabis users [6]. The impaction filter device used here successfully collected THC from cannabis users, suggesting that THC is carried in breath aerosols. However, THC in breath at 1 h to 1.5 h post-use was not necessarily higher than at baseline, even when THC in blood increased at least five-fold immediately post-use, indicating compliance with the protocol. This may be related to differences in breath sampling, as participants may have found the breathing maneuver more challenging to execute when intoxicated. Further investigation is required to identify factors that lead to outliers based on sampling differences.

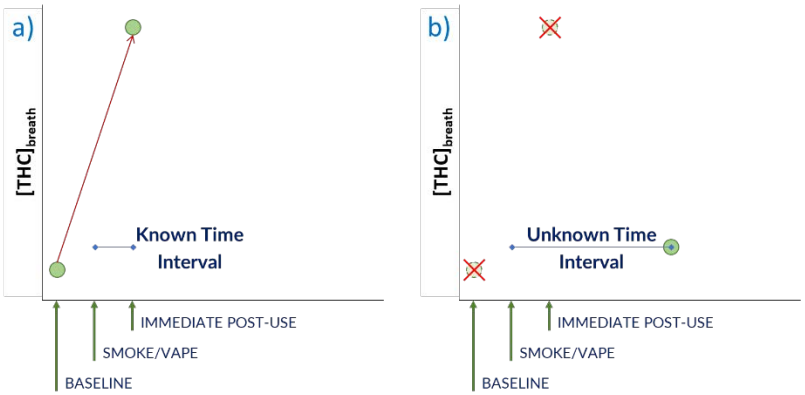

Figure 3. Comparison of the evaluation strategy for protype devices (a) vs. the reality at the roadside (b) in which there is no baseline measurement, no immediate post-use measurement, and an unknown time interval has elapsed between smoking/vaping and breath sampling.

When measured from 1 h to 1.5 h after cannabis use, $[THC]_{breath}$ values, reported in ng/device, varied over orders of magnitude in a series of published, pilot-scale studies (Figure 4). In these studies, breath was sampled with filter-based devices and THC (and sometimes other cannabinoids) was subsequently extracted, concentrated, and analyzed with laboratory instruments to quantify THC at baseline and at various post-cannabis use time points. We used the BreathExplor device (impaction filter) [2]. Lynch et al. used the HoundLabs device (packed bed plus electrostatic filter) [17]. All other studies used the ExaBreath device (electrostatic filter) including two studies not included in the figure [18,19]. For the studies summarized in Figure 4, there were four participants with no THC in their post-use breath extracts. In contrast, Hubbard et al. [18] reported that only 37% of participants had THC in their breath extracts at the second post-use time point, which was collected 40 min to 90 min after cannabis use. Fitzgerald et al. [19] similarly reported a low fraction of participants with THC in their breath 90 min after cannabis use. Both studies used the ExaBreath (SensAbues) device. For the studies summarized in Figure 4, many $[THC]_{breath}$ values fell within 0.02 ng/device and 2 ng/device (dashed red lines), but $[THC]_{breath}$ values an order of magnitude higher were also observed, indicating a challenge for breathalyzer development.

It is important to note that the studies summarized in Figure 4, and the Hubbard et al. [18] and Fitzgerald et al. [19] studies, had a variety of differences, including differences in the study populations (i.e., daily vs. occasional cannabis use), abstinence instructions (if any), cannabis product, cannabis use during the study session, and breath sampling protocol. Whether or not these differences might be expected to produce meaningful differences in $[THC]_{breath}$ requires studies with statistically relevant participant numbers. Consistent with other peer-reviewed studies, our results do not support the idea that a single timepoint measurement of THC in breath can, at present, reliably indicate recent cannabis use.

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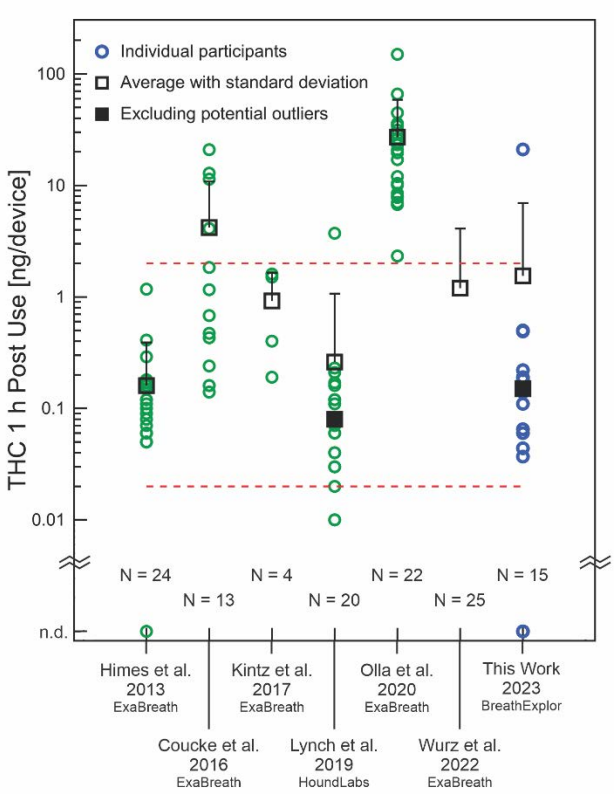

Figure 4. Comparison of THC (ng/device) recovered approximately 1 h after cannabis use with ExaBreath (electrostatic filter), HoundLabs (packed bed plus electrostatic filter), or BreathExplor (impaction filter) devices. Sample size (N) indicates the number of participants who completed this specific post-use timepoint. Himes et al. [20] sampled breath between 0.7 h and 1.1 h after use and Olla et al. [21] sampled breath 1.5 h after use, while we [2] sampled breath between 1.0 h and 1.5 h after use. Other studies reported sampling breath 1.0 h after use. Wurz et al. [22] did not provide measurements for individual participants; the average and standard deviation provided here are based on figure digitization. Dashed red lines at 2 ng/device and 0.02 ng/device are to guide the eye.

New DVME method is capable of vapor pressure measurements with state-of-the-art measurement uncertainty on large molecules in as little as 15 min. DVME performance was first validated with p^{sat} measurements on the reference compound n-eicosane ($C_{20}H_{42}$). As mentioned earlier, one of the reasons for starting with n-eicosane is that it has the lowest-uncertainty p^{sat} measurements and correlations (p^{corr}) for any molecule its size [13]; thus, it is the best choice for identifying systematic measurement errors and for confirming our uncertainty estimates. DVME p^{sat} measurements of n-eicosane from 344 K to 374 K ranged from 0.452 Pa to 5.74 Pa and were compared with published measurements and models (Figure 5). Tables of data for the points shown in Figure 5 are available in [3]. The excellent agreement between our measurements and the reference correlation of Lemmon and Goodwin (represented by the black line in Figure 5) demonstrates the high quality of our data. The reference correlation has an estimated uncertainty of ≥3.4%, while our measurements have estimated uncertainties ranging from 2.0%

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to 2.8%, though encompassing a narrower temperature range. DVME p^{sat} measurements deviate from p^{corr} by an average of 1.4% and a maximum of 2.9%, which is less than the uncertainty in the reference correlation itself. DVME delivers state-of-the-art measurement uncertainty for this p^{sat} range.

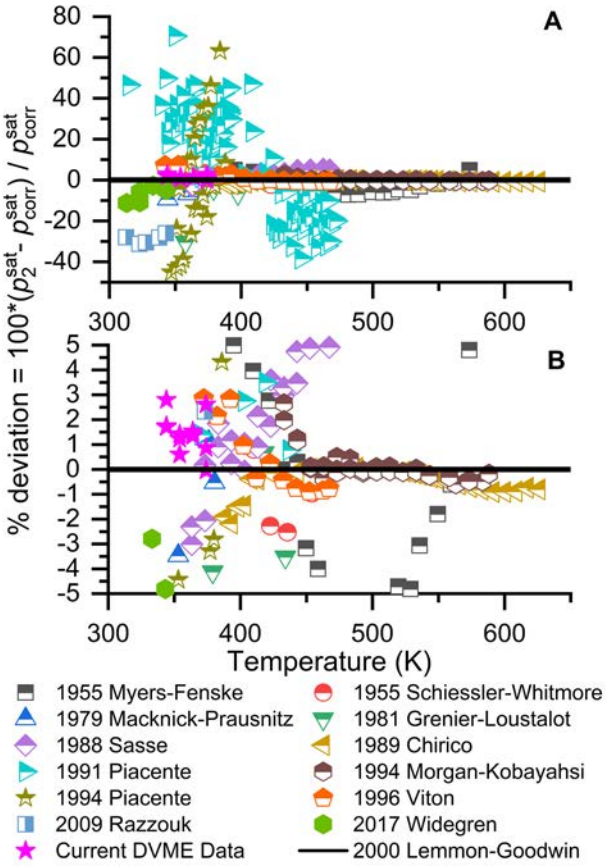

Figure 5. Percent deviation of current and previous p^{sat} measurements of n-eicosane from the reference correlation of Lemmon and Goodwin [13]. All experimental data points are shown in panel (A). The y-axis expansion in panel (B) shows data within 5 % of the reference correlation. DVME measurements are represented by pink stars.

Control experiments were performed to better understand the influence of the thermal equilibration period, the carrier gas flow rate, and the total carrier gas flow on the measured p^{sat} value of n-eicosane. These experiments were performed at 364 K, the highest temperature used in the study, because higher (and lower) temperatures are more challenging from the standpoint of thermal equilibration. This limitation will be investigated in future developments. The measured p^{sat} value was independent of carrier gas flowrate over the range of the mass flow meter, which is 0.5 to 10 standard cubic centimeters per minute (sccm). With a flowrate of 10 sccm, the measured p^{sat} reached a steady value at total flows ≥ 120 scc and thermal equilibration periods ≥ 3 min. Thus, a total measurement period as short as 15 min (3 min of thermal

equilibration plus 12 min of carrier gas flow) is sufficient for high-quality p^{sat} measurements at temperatures up to 364 K.

New DVME method is capable of vapor pressure measurements over a wide pressure range and with unstable molecules. DVME performance was next evaluated with p^{sat} measurements on the cannabis-associated terpene linalool [4]. Although not of the same quality or quantity as for n-eicosane, reliable p^{sat} measurements do exist for linalool [14]. This was important because the DVME method had not yet been proven reliable for compounds less stable than alkanes. Given that linalool is also much more volatile than n-eicosane, two simple modifications of the apparatus were needed: the diameter of the capillary vapor trap was increased, and a thermoelectric plate was added to cool the capillary vapor trap.

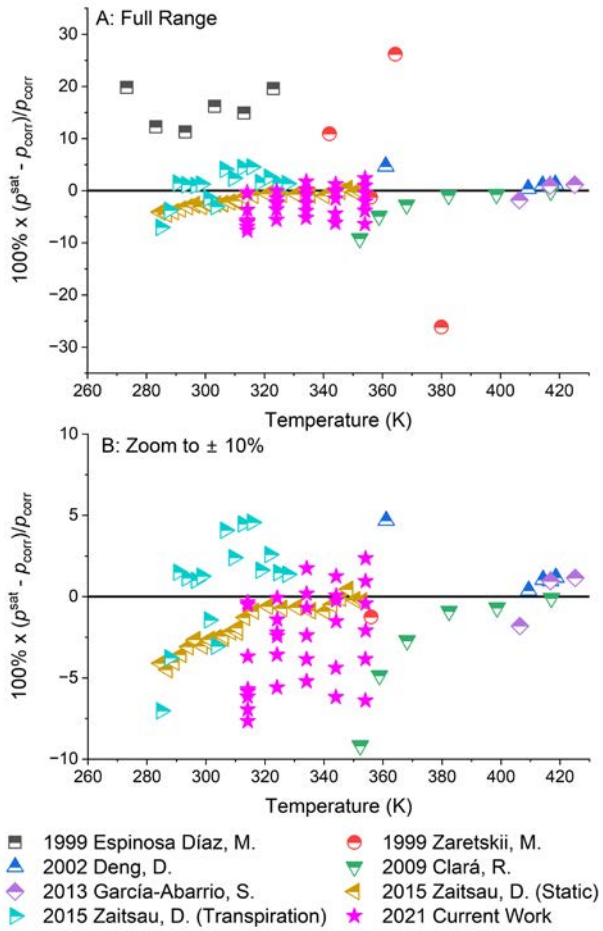

Figure 6. Percent deviation of current and previous p^{sat} measurements of linalool from a Wagner correlation of all previously published data between 270 K to 425 K [4]. All experimental data points are shown in panel (A). The y-axis expansion in panel (B) shows data within 10 % of the correlation. DVME measurements are represented by the pink stars.

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DVME p^{sat} measurements of linalool from 314 K to 354 K ranged from 81 Pa to 1250 Pa and were compared with published measurements (Figure 6). Tables of data for the points shown in Figure 6 are available in [4]. Note that the black line in Figure 6 is a Wagner correlation of all available literature data from 270 K to 425 K and, as such, does not represent a reference correlation. However, the correlation is heavily influenced by two data sets from Zaitsau et al. (gold triangles and teal triangles) because of their numerous data points. The high quality of the data from Zaitsau et al. is indicated by the relatively small scatter and the good agreement obtained with two different measurement methods: a transpiration method and a static method [14]. The transpiration data, on average, are a little higher and a little more scattered than the static data, but it is not clear which data set is more accurate. In any case, our measurements overlap the data from Zaitsau et al. and have a similar level of scatter as their transpiration data. Additionally, for 22 of the 32 data points, DVME p^{sat} measurements deviate from the Wagner correlation by less than their combined standard uncertainty. Such agreement is evidence that our uncertainty estimates are reasonable, and shows that DVME can deliver accurate results for p^{sat} values 200 times higher than n-eicosane.

Although the measurement period is relatively short, an advantage for p^{sat} measurements of compounds that are less stable than alkanes, three additional precautions were taken. First, to minimize the potential for oxidative decomposition, 0.2 mass % of the antioxidant tert-butylhydroquinone (TBHQ) was added to the linalool before the p^{sat} measurements [23]. The specific decomposition pathway of concern is the autoxidation of the C=C double bonds (this moiety is common in terpenes and cannabinoids). Second, measurements were made from the lowest to the highest temperature, then the initial 314 K (lowest) temperature was repeated at the end of the measurement series. These replicate data points were all within 3.2 % of the initial values, less than the combined standard uncertainty of the linalool measurements, which demonstrates that any decomposition of linalool was insignificant from the standpoint of the p^{sat} measurement. Third, a given saturator vial was used for only a single day before replacement (5 or 6 measurements). With these precautions in place, no evidence of linalool decomposition was observed by GC-FID in the trapped linalool or in the linalool remaining in the saturator vial after a complete series of p^{sat} measurements. DVME is thus capable of p^{sat} measurements on unstable molecules.

Fluid flowrate has a dramatic effect on the deposition of aerosols in an impaction filter and both interception and impaction mechanisms are possible, depending on flowrate. Computational fluid dynamics simulations of the normalized fluid velocity along the y axis of the impaction filter at 0.4 L/s (Figure 7) reveals up to 11x local velocity enhancement compared to the initial velocity; these regions are blue/violet and are located near the baffle edges. However, the red regions show that there are also regions of low or negative (upstream) velocity, where the fluid is either stagnant or directed towards the inlet. The regions of enhanced or diminished velocity are consistent in magnitude for all flowrates examined and, as

such, are induced primarily by the abrupt geometric changes of the baffle arrangement and enhanced by turbulent eddies.

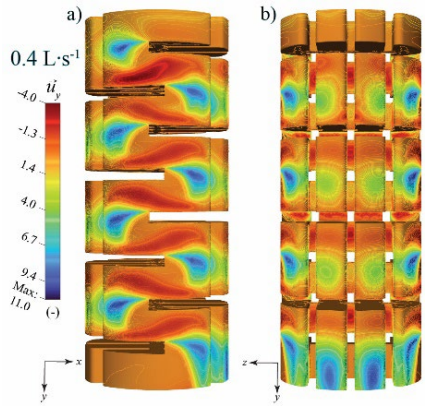

Figure 7. Two dimensional views of the normalized velocity for an inlet velocity of 0.4 L/s. Both the (a) xy plane and (b) yz plane of the filter are shown and the inlet is at the top of each filter. In actual use, the filter orientation would be horizontal (y axis oriented parallel to the earth).

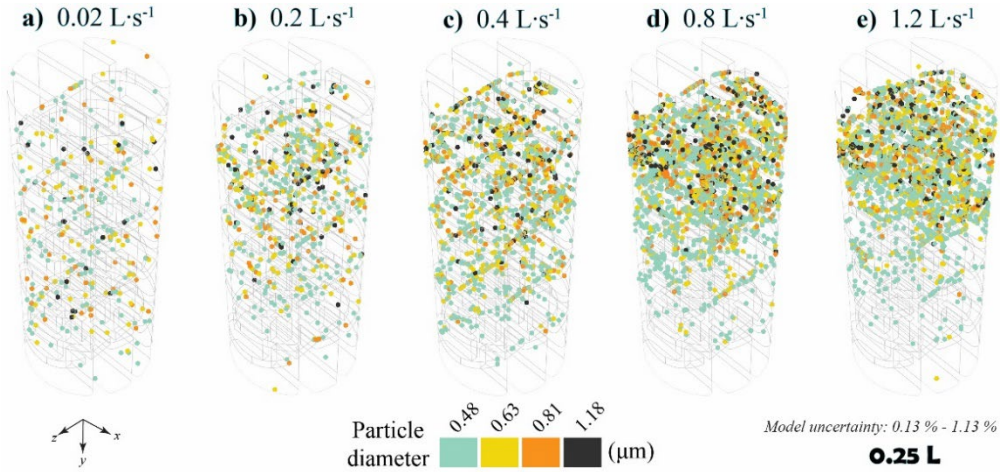

Figure 8. Particle deposition as a function of particle diameter after 0.25 L of fluid volume passes through the filter for flowrates of a) 0.02 L/s, b) 0.2 L/s, c) 0.4 L/s, d) 0.8 L/s, and e) 1.2 L/s. Note that the particles are not to scale and all are represented with the same dot size.

Computational fluid particle dynamics simulations were used to examine mass deposition after 0.01 L, 0.125 L, 0.25 L, and 2 L of fluid volume through the filter at a flowrate of 0.4 L/s (data not shown). After 0.01 L of fluid volume, the mass deposition was 72.8 %, whereas for the other fluid volumes (0.125 L, 0.25 L, and 2 L), there was a consistent mass deposition of 93.6 %, indicating that the simulation had reached equilibrium by 0.125 L. Accordingly, to examine particle deposition and distribution for all flowrates, we simulated 0.25 L of fluid volume through the filter (Figure 8), selecting a representative fraction of the deposited particles for visualization. At the lowest flowrate (0.02 L/s), low velocities hinder the deposition of even the largest particles and particles are relatively uniformly distributed in the filter. Particles travel

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along fluid flowlines and the primary deposition mechanism is interception. At the highest flowrates (0.8 L/s and 1.2 L/s), high velocities promote the deposition of particles within the first third of the filter, particularly for the largest particles, and the primary deposition mechanism is impaction.

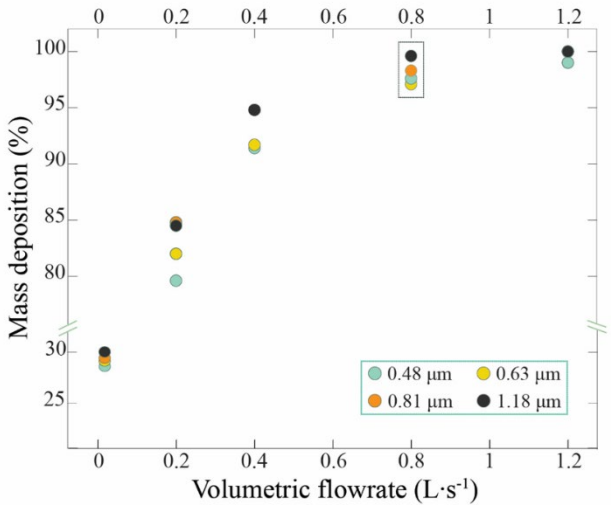

Figure 9. Mass deposition as a function of volumetric flowrate and particle diameter after 0.25 L of fluid volume passes through the filter. Note that the y-axis is split to display the low mass deposition at 0.02 L/s on the same plot.

The contribution of flowrate to mass deposition is dramatic (Figure 9). While flowrates of 0.8 L/s and 1.2 L/s result in nearly 100% mass deposition, deposition decreases with flowrate to 90-95% by mass (0.4 L/s) and 80-85% by mass (0.2 L/s), and is only 30% by mass at 0.02 L/s. While the highest flowrates are unlikely to be achievable or sustainable for most individuals, intermediate flowrates could be observed in realistic breath sampling scenarios. These flowrates correspond to exhalation flowrates of 0.6 L/s and 1.2 L/s, and flowrates measured by spirometry are likely to fall at the lower end of this range, or even lower, if flowrate is not specified. Therefore, specifying a minimum exhalation flowrate should improve the fraction of aerosols captured with the BreathExplor device and the consistency of samples collected from different individuals or on different days. As a starting point, the exhalation flowrate and volume should be measured.

Limitations

Our pilot breath sampling study used a cost-effective mechanism to “piggy back” breath sampling onto a separately funded study of cannabis use. This meant that only a subset of participants (i.e., those who were assigned to the THC-dominant cannabis flower product) were invited to participate in the cannabis breathalyzer study, which had a separate informed consent process. This created several limitations. First, the breath sampling timepoints were limited, as might be expected. Second, some participants who were

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recruited into the anxiety study were opposed to participating in a cannabis breathalyzer study. Third, complete sets of breath samples were frequently not collected because participants could opt in/opt out separately. **These limitations can be addressed by human studies explicitly designed to evaluate or improve breath-based measurements.** Vapor pressure measurements near breath temperature for semi-volatile compounds like cannabinoids and terpenoids remain a significant challenge for all existing gas-saturation methods. While low-uncertainty measurements such as those achieved here can be used to extrapolate to breath temperature or develop an equation of state, experimental values are superior because of the many assumptions that go into prediction. **This limitation can be addressed by improving temperature stability near ambient with further apparatus development.** Our simulations of fluid flow and sub-micron particle deposition did not include the entire device, which consists of a mouthpiece followed by three impaction filters in parallel. While simulating a single filter does not capture the full complexity of the device, it was an efficient way to begin examining the effect of human factors on aerosol capture for use in future human study designs. **This limitation can be addressed through future simulations that examine the effect of the mouthpiece on flowrate and particle deposition.** Together, human subject studies, low-uncertainty laboratory measurements, and numerical simulations are a powerful combination of tools that can be deployed to uncover and mitigate sources of scatter towards a meaningful measurement of recent cannabis use.

Artifacts

Peer-reviewed Publications

1. M.E. Harries, C.N. Beuning, B.L. Johnston, T.M. Lovestead, J.A. Widegren**.** Rapid vapor-collection method for vapor pressure measurements of low-volatility compounds. *Analytical Chemistry* (**2020**). https://doi.org/10.1021/acs.analchem.0c04131

2. K.M. Jeerage, C.N. Beuning, A.J. Friss, L.C. Bidwell, T.M. Lovestead. THC in breath aerosols collected with an impaction filter device before and after legal-market product inhalation - a pilot study. *Journal of Breath Research* (**2023**). https://doi.org/10.1088/1752-7163/acd410

3. **ACS Editor's Choice** C.N. Beuning, T.M. Lovestead, M.L. Huber, J.A. Widegren. Vapor pressure measurements on linalool using a rapid and inexpensive method suitable for cannabis-associated terpenes. *Journal of Chemical and Engineering Data* (**2023**). https://doi.org/10.1021/acs.jced.3c00360

4. V.D. Malavé, K.M. Jeerage, E.J. Garboczi, T.M. Lovestead. 3D computational fluid and particle dynamics simulations: metrics of aerosol capture by impaction filters. *Journal of Breath Research* (**2024**). https://doi.org/10.1088/1752-7163/acfe32

Data Sets

None.

Dissemination Activities (only the presenting author is indicated)

1. C.N. Beuning. Rapid vapor collection method for vapor pressure measurements of low-volatility compounds. *Oral presentation at the American Chemical Society (ACS) Rocky Mountain Regional Meeting, Virtual, 2020.*

2. T.M. Lovestead. Chemical foundations for a cannabis breathalyzer: vapor pressure measurements and a pilot breath collection study" *Oral presentation at the NIJ Research Symposium, Virtual, 2021.*

3. K.M. Jeerage "Partitioning relationships to quantitate organic molecules in air or breath samples" *Oral presentation at the 73nd Meeting of the American Academy of Forensic Sciences (AAFS), Virtual, 2021.*

4. J.A. Widegren. Thermodynamic measurements that provide ground truth in forensic science: applications to breath and headspace analysis. *Invited oral presentation at PITTCON, Virtual, 2021.*

5. T.M. Lovestead**.** Chemical foundations for a cannabis breathalyzer: vapor pressure measurements and a pilot breath collection study. *Oral presentation at PITTCON, Virtual, 2021.*

6. C.N. Beuning. Obtaining small uncertainty in vapor pressure measurements of low volatility and low stability compounds with rapid dynamic vapor microextraction. *Oral presentation at the 21st Symposium on Thermophysical Properties, Virtual, 2021.*

7. T.M. Lovestead. The role of thermophysical properties measurements in advancing forensic science. *Oral presentation at the 21st Symposium on Thermophysical Properties, Virtual, 2021.*

8. K.M. Jeerage. Challenges in reproducible capture and analysis of cannabinoids with an impaction filter device. *Oral presentation at the 74nd Meeting of the AAFS, Seattle, 2022.*

9. C.N. Beuning. Quantitative analysis of cannabinoids in exhaled breath aerosols by high-performance liquid chromatography/electrospray ionization-tandem mass spectrometry (HPLC/ESI-MS/MS). *Poster presentation at the 74nd Meeting of the AAFS, Seattle, 2022.*

10. T.M. Lovestead. Building the chemical foundation necessary for a reliable cannabis breathalyzer. *Poster presentation at the NIJ Research Symposium, Virtual, 2022.*

11. C.N. Beuning. Obtaining small uncertainty in vapor pressure measurements of eicosane and linalool with rapid dynamic vapor microextraction. *Invited poster presentation at SCIMIX at the ACS Spring Meeting, San Diego, 2022.*

12. C.N. Beuning. Quantitative analysis of cannabinoids in exhaled breath aerosols by HPLC ESI-QQQ-MS/MS. *Invited poster presentation at SCIMIX at the ACS Spring Meeting, San Diego, 2022.*

13. C.N. Beuning. Obtaining small uncertainty in vapor pressure measurements of eicosane and linalool with rapid dynamic vapor microextraction. *Poster presentation at the ACS Spring Meeting, San Diego, 2022.*

14. C.N. Beuning. Quantitative analysis of cannabinoids in exhaled breath aerosols by HPLC ESI-QQQ-MS/MS. *Oral presentation at the ACS Spring Meeting, San Diego, 2022.*

15. T.M. Lovestead. *Invited seminar at the University of Iowa, 2022.*

16. C.N. Beuning. *Invited seminar at the Colorado ACS Meeting, 2022.*

17. T.M. Lovestead. Chemical foundations for a cannabis breathalyzer. *Poster presentation at International Association of Breath Research (IABR) Breath Summit, Pisa, 2022.*

18. K.M. Jeerage. Capture and analysis of cannabinoids with an impaction filter device. *Oral presentation at the International Council on Alcohol, Drugs & Traffic Safety (ICADTS), Rotterdam, 2022.*

19. V.D. Malavé. *Invited seminar at the University of Colorado – Colorado Springs, 2022.*

20. K.M. Jeerage. Developing the infrastructure for reliable cannabis breathalyzer devices. *Invited oral presentation at Forensics @ NIST, Virtual, 2022.*

21. V.D. Malavé. Clinical and forensic characterization of exhaled breath: CFPD simulations of the metrics of aerosol particle capture. *Invited oral presentation at the International Congress on Advanced Materials Science and Engineering (AMSE), Vienna, 2023.*

22. T.M. Lovestead. Building the chemical foundation necessary for a meaningful cannabis breathalyzer. *Invited oral presentation at PITTCON, Philadelphia, 2023.*

23. V.D. Malavé. Clinical and forensic characterization of exhaled breath: computational modeling for the metrics of aerosol particle capture. *Oral presentation at the 17th U.S. National Congress on Computational Mechanics, Albuquerque, 2023.*

24. V.D. Malavé. Characterization of exhaled breath – multidisciplinary computational studies for the metrics of aerosol transport and deposition. *Oral presentation at the 8th International Conference of Particle-Based Methods, Milan, 2023.*

25. K.M. Jeerage. Human factors may influence THC capture with an impaction filter device: towards standardized sampling and calibration protocols. *Poster presentation at the Society of Forensic Toxicologists (SOFT) Meeting, Denver, 2023.*

Dissemination Activities for General Audiences

1. R. Press. Researchers analyze THC in breath of cannabis smokers. *NIST News Release*, May 22, 2023.

2. B. Adlin. Breath testing is not yet a reliable indicator of recent marijuana use, federal study finds. *Marijuana Moment*, May 23, 2023.

3. L. Bear-McGuiness. Cannabis breathalyzers may not be possible, NIST study suggests. *Analytical Cannabis*, May 24, 2023.

4. M. Benz. Cannabis breathalyzer still in its infancy: Interview with Tara Lovestead. *Medical Research Com*, May 24, 2023.

5. H. Johnston. Sniffing out drugged driving: why a breath test for cannabis is so hard to create: Interview with Kavita Jeerage. *Physics World Weekly Podcast*, June 22, 2023.

6. L. Marshall. A reliable cannabis breathalyzer? Possible, but not easy. *CU Boulder Today*, Sept 11, 2023.

7. O. Doak. CU Boulder tests science behind creating a cannabis breathalyzer. *Boulder Daily Camera*, Oct 27, 2023.

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