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Development of a semi-automated volatile organic compounds (VOCs) sampling system for field asymmetric ion mobility spectrometry (FAIMS) analysis

Milton Valencia-Ortiz, Sindhuja Sankaran *

Department of Biological System Engineering, Washington State University, Pullman, WA 99164, USA

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ABSTRACT

In recent years, applications of volatile organic compounds (VOCs) sensing technologies such as field asymmetric-waveform ion-mobility spectrometry (FAIMS) system in agriculture have accelerated. FAIMS system for VOCs sensing is attractive as it offers high sensitivity, selectivity, real-time monitoring, and portability. However, the development of a robust instrumentation system is needed for precise sampling, high accumulation of VOCs, and careful handling of samples. In this study, we developed a simple semi-automated VOC sampling (SAVS) system using a Raspberry Pi microcontroller, flowmeters, electromechanical solenoid, and cellphone-based app to control cleaning and sampling loops. The system was compared with customized headspace sampling apparatus (CHSA) and validated with a biomarker (acetone) identified to be associated with potato rot development during postharvest storage. The standard error within ion current data across different compensation voltage was lower using the SAVS system than the CHSA. In addition, the maximum peak values across scans displayed a high coefficient of variation using the CHSA (16.23%) than the SAVS system (4.51%). Future work will involve improving system efficiency by adapting multiple sample units, system miniaturization, and automating the flowmeter operation. Such automation is critical to characterize VOCs precisely and automatically across several samples for multiple applications such as pathogen detection, evaluation of crop responses, etc.

Specifications table

Hardware name	Semi-automated VOCs sampling (SAVS) system
Subject area	Biological Sciences (e.g., Microbiology and Biochemistry) General
Hardware type	Biological sample handling and preparation

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* Corresponding author.
E-mail addresses: m.valenciaortiz@wsu.edu (M. Valencia-Ortiz), sindhuja.sankaran@wsu.edu (S. Sankaran).

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Hardware name	Semi-automated VOCs sampling (SAVS) system
Closest commercial analog	There was a sampling system (ATLAS Headspace Sampler) developed by Ownstone Medical Ltd., which was strictly used for clinical samples such as urine, stool, saliva, sweat and blood. Besides, the fabrication of this system was discontinued and is no longer available to purchase since September 2021 (https://www.owlstonemedical.com/products/atlas/).
Open-source license	*GNU General Public License (GPL)*
Source file repository	*None to report. Programming codes have been cited.*

Hardware in context

Plant-emitted VOCs play an essential role in many ecological functions [1]. These functions include defending themselves from biotic and abiotic factors, providing information and potential misinformation to mutualists and competitors [2], and promoting plant growth [3]. Various defense pathways are activated, resulting in the induction of a complex blend of volatiles, that include key volatiles such as E-2-hexenal [4], (E)-Nerolidol [5], methyl salicylate, indole, and sesquiterpenes [6]. The association between these volatiles and plant pathogen defense mechanism makes these compounds the ideal candidates for use as biomarkers for plant phenotyping applications [7–9]. For example, in *Vitis vinifera*, the response to infection with the fungus *Phaeoacremonium parasiticum* induces the biosynthesis of nerolidol (phytoalexin). This VOC was found to reduce fungal growth by inhibiting mycelial growth [10].

The detection and quantification of VOCs are difficult due to their intrinsic reactivity and low concentrations [11] and the need for expensive equipment (e.g., gas chromatography – mass spectrometry/GC–MS, proton transfer reaction – mass spectrometry/PTR-MS). Therefore, asymmetric-waveform ion mobility spectrometer (FAIMS) system and similar high-throughput volatile sensing technologies have emerged as an affordable and complementary technologies to GC–MS and PTR-MS systems for monitoring VOC profiles in laboratories, controlled environment, and field conditions. Sensitivity, selectivity, field portability, analytical flexibility, and real-time tracking are the main attributes of FAIMS system that make it desirable for agricultural applications [12]. Further, FAIMS system allows for customization, flexible sampling with different types of units or systems, and ideal for different biological samples (such as potato tuber, grain, stem, leaf, flower, and soil). FAIMS technology has sampling systems such as ATLAS headspace sampler [13,14] or Breath Biopsy® Collection Station [15], which is limited by small sample size or specific biomedical application. Therefore, this study, focuses on the development of sampling system for diverse biological samples.

In recent years, FAIMS system has been used in various agricultural research studies. Particularly in post-harvest storage, where rot diseases can cause significant loss of potato tubers. For example, FAIMS system demonstrated the ability to detect soft rot at early stages in potatoes [16–20]. In onion, FAIMS system also demonstrated its applicability for onion sour skin detection under storage conditions [18,21]. In these studies, the VOCs sampling relies on an existing customized headspace sampling apparatus (CHSA) modified by Sinha, et al. [14], which was manually operated and adapted to collect VOCs from a sample chamber. CHSA works as a dilution chamber that encloses the samples and facilitates the volatile movement to the FAIMS system detector. In other studies, the dilution chamber is presented with additional elements such as a flowmeter and drying tube [22] and/or a photocatalysis reactor [23]. However, these developments lack an automation process for online analysis of VOCs from sample headspace, though some developments are available in other types of samples [24,25]. In addition, the common sampling technique (e.g., solid-phase microextraction, stir-bar sorptive extraction, silicon-based tubing) are designed for centralized facilities (e.g., GC–MS, PTR-MS). Thus, fast and reliable VOCs sampling methodology for systems such as FAIMS technology is needed, especially for agricultural applications.

To illustrate and compare the manual sampling of VOCs, here we detail the characteristics of the CHSA. The CHSA was made up of a glass chamber (3.81 L) and one Teflon stopper with two holes that were sealed with 3 cm of Teflon tubes using push-to-connect tube fitting (Fig. 1). The CHSA application relies on a fully manual operation consisting of the following steps: First, the sample is introduced. Second, the stopper is tightly sealed. Third, the stopper sealed tubes are removed to connect two Teflon tubes; one from incoming carries gas (air or nitrogen) and the second one to push the sample VOCs to the FAIMS system. Fourth, the tank of carrying gas is opened until the CHSA is pressurized, which takes around 90 *sec* to reach 1.6 L/min at 60000 Pa. Finally, the FAIMS system is used to collect sample headspace VOCs profile scans. The third and fourth steps from the above mentioned CHSA operation can lead to losses of VOCs from the sample headspace prior to detection using the FAIMS system. Ideally, the tubes connections should be made as quickly as possible and simultaneously, which is challenging during the manual operation. Also, during sample pressurization, the VOCs maybe lost through the FAIMS system exhaust valve. These conditions can result in inconsistent VOCs profiles. In addition, the loss becomes very critical if the VOCs are present in low concentration. To address these issues and avoid the VOCs losses, we developed a semi-automated VOCs sampling (SAVS) system that integrated the CHSA with the FAIMS system in an efficient manner. This system includes independent sample pressurization, rapid stabilization of gas flow (at a constant rate and pressure), and automated switching between sampling and purging/cleaning with the FAIMS system. The functionality was validated with acetone, commonly detected from rot diseases in stored potatoes [26].

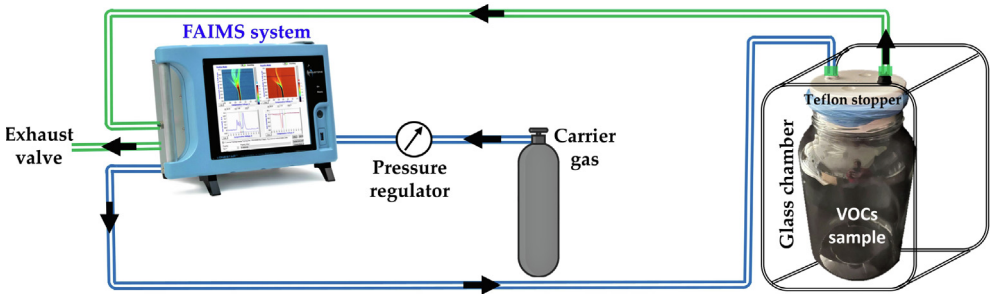

Fig. 1. Customized headspace sampling apparatus (CHSA) integrated with the FAIMS system. (Adopted and edited from Sinha, et al. [17]).

Hardware description

As stated in above section, the existing sampling method (CHSA, Fig. 1) relies completely on manual operation within a single loop (sampling), which can lead to VOCs losses during connection of tubes and sample pressurization. Thus, the SAVS system was developed to address these problems by using four main components (Raspberry Pi 3 + microcontroller, two electro-mechanical solenoids valves, two gas flowmeters, and a mobile application to control the solenoids valves). Two gas flow loops were identified to address the semi-automation functions. The first loop was called "cleaning loop" (also referred to as purging), which is a critical procedure to remove the residual VOCs from previous samples in the system to avoid cross contamination. The second loop was called "sampling loop", this loop was designed to achieve three functions, which include sample preparation, pressurization, and sampling of VOCs from sample headspace. Sample preparation and pressurization can be done at the same time as the cleaning loop (when the FAIMS system is being cleaned). Once the FAIMS system is cleaned and sample is pressurized, the sampling function is activated. Both cleaning and sampling loops provide a ease in the workflow (FAIMS system cleaning, sample preparation, sample pressurization, and sampling) that allow consistent VOCs profiles data collection. The components of the SAVS system can be commercially acquired, and their assembly is relatively easy. These characteristics enable their future replication, as well as the flexibility to develop and use multiple CHSA units.

SAVS system is a semi-automated VOCs sampling system that enable an automated control of sampling and cleaning loops using a mobile app, which can be integrated with a FAIMS system or similar units. During the cleaning loop, the user can manually prepare the sample and pressurized it, which will allow rapid stabilization of sampling conditions (pressure and flow rate) during sampling loop.

Design files summary

The design is included in the manuscript.

Bill of materials summary

Designator	Component	Amount	Source of materials	Image
*P*1	2 Channel 5 V Relay Module	1	Amazon	
*P*2	Brass Push-to-Connect Tube Fitting for Air, Tee Connector, for 1/8″ Tube OD	2	McMaster-Carr	
*P*3	Brass Push-to-Connect Tube Fitting for Air, Straight Connector, for 1/8″ Tube OD	1	McMaster-Carr	

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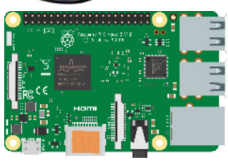
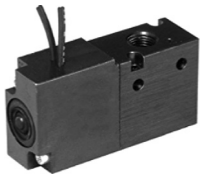
Designator	Component	Amount	Source of materials	Image
*P*4	Push-To-Connect Tube Fitting for Air, Straight Adapter, For 1/8″ Tube OD × 1/8 NPT Male	9	McMaster-Carr	
P5	Extreme-Temp Teflon PTFE Semi-Clear Tubing for Chemicals, 1/16″ ID, 1/8″ OD, 25 ft. Length	1	McMaster-Carr	
*P*6	High-Pressure Inline Filter, 303 Stainless Steel Housing, 1/8 Npt Female × Male, 10 μm	4	McMaster-Carr	
P7	12 V AC/DC Adapter	3	Amazon	
P8	Raspberry Pi 3	1	Pishop	
P9	2-Position, 3-Way Body Ported Solenoid	2	Cole-Parmer	
P10	Masterflex Proportional Flowmeter Controller, Mass; 5 L/min Gas	2	Masterflex	
P11	Unfinished Wooden Square Shape. 0. 3 × 0.3 *m*	1	Local hardware store	

Build instructions

SAVS system assembly

A microcontroller, solenoids, and flowmeters were attached to a wooden board using hook and loop fasteners (e.g., Velcro) in pre-cut strips. Teflon tubes were used to connect all the elements using push-to-connect tube fittings. The assembly protocol consisted of two main parts as described below.

Mechanical assembly

Mechanical assembly was divided into two physical loops: First, the cleaning loop provides the connections and air flow for FAIMS system purging or cleaning (Fig. 2 and Fig. 3a, b). This loop required a tank or flow of carrier gas (Fig. 2a) connected to an internal filter from the FAIMS system, and the filtered gas was regulated with a high precision flowmeter (Fig. 2b). The outgoing gas was channelized up to the FAIMS system through a 3-way electromechanical solenoid valve and Tee-connector (Fig. 2c, d, e). The second loop provides a sampling loop—this loop was connected to the cleaning loop through the aforementioned 3-way solenoid valve and Tee-connector. The sampling loop required a second gas tank or carrier gas flow (Fig. 6f) for sample pressurization, and it was regulated with a second flowmeter (Fig. 2g). The outgoing gas was channelized with the CHSA through Tee-connector (Fig. 2h, i). The CHSA outgoing gas (Fig. 2j) was channelized up to the FAIMS system through a 3-way electromechanical solenoid valve (Fig. 2k) that connected with the Tee-connector from the cleaning loop (Fig. 2d).

Electronic assembly, software installation, and remote control

Assembling the electronics enabled the semi-automation of the cleaning loop, sample preparation, sample pressurization, and sampling loop (Fig. 3). First, a Raspberry Pi microcontroller (Pi compute module 3 + lite with Cortex-A53, ARMv8, 64-bit at 1.2 GHz processor, and 1 GB RAM) and a two-channel relay (Fig. 2l) were installed to control the electromechanical solenoid valve (Fig. 2c, h). Pin 2 (5 V), 6 (ground), 11 (General-purpose input/output GPIO 17), and 12 (GPIO 18) were used to establish the connections between the microcontroller and two-channel relay (Fig. 2m). Next, the two-channel relay was connected to the solenoid valves using common terminals connections and voltage terminal NO (normally open) with an external power supply (Fig. 2n). Finally, a bridge connection was placed between the NO terminal (Fig. 2o, white wire).

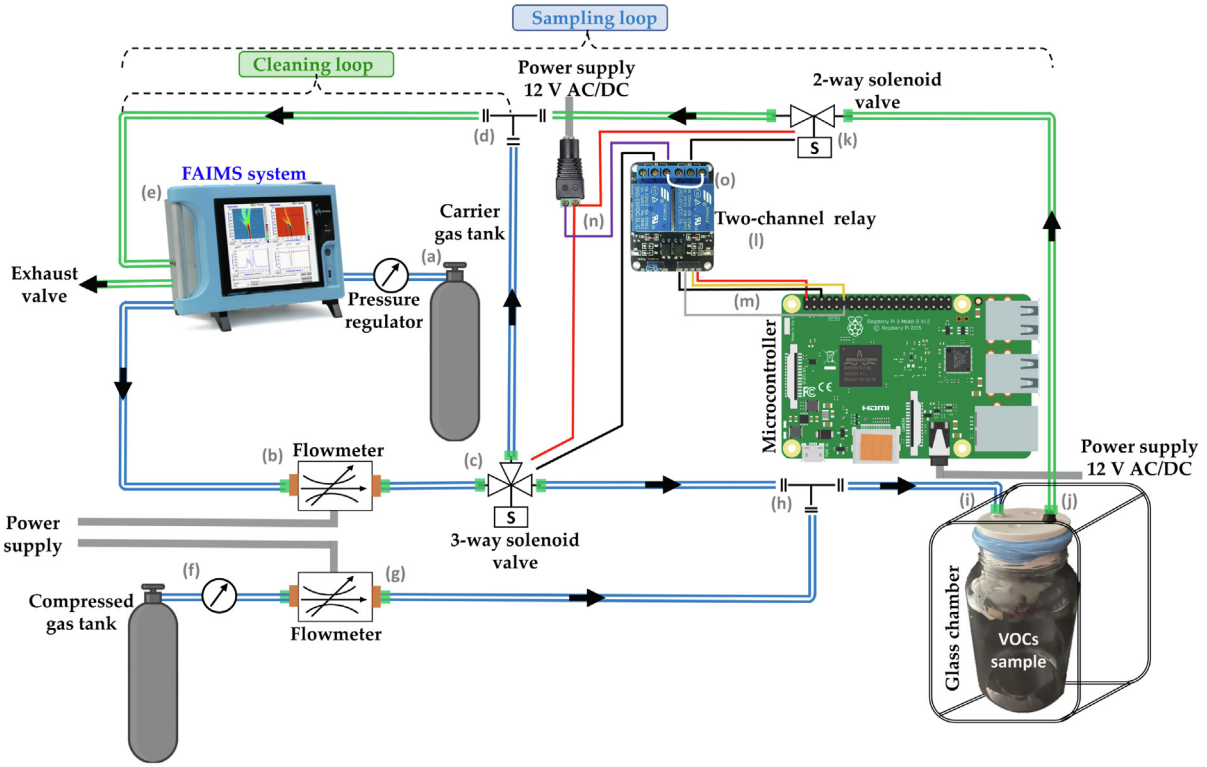


Fig. 2. Semi-automated VOCs sampling (SAVS) system design.

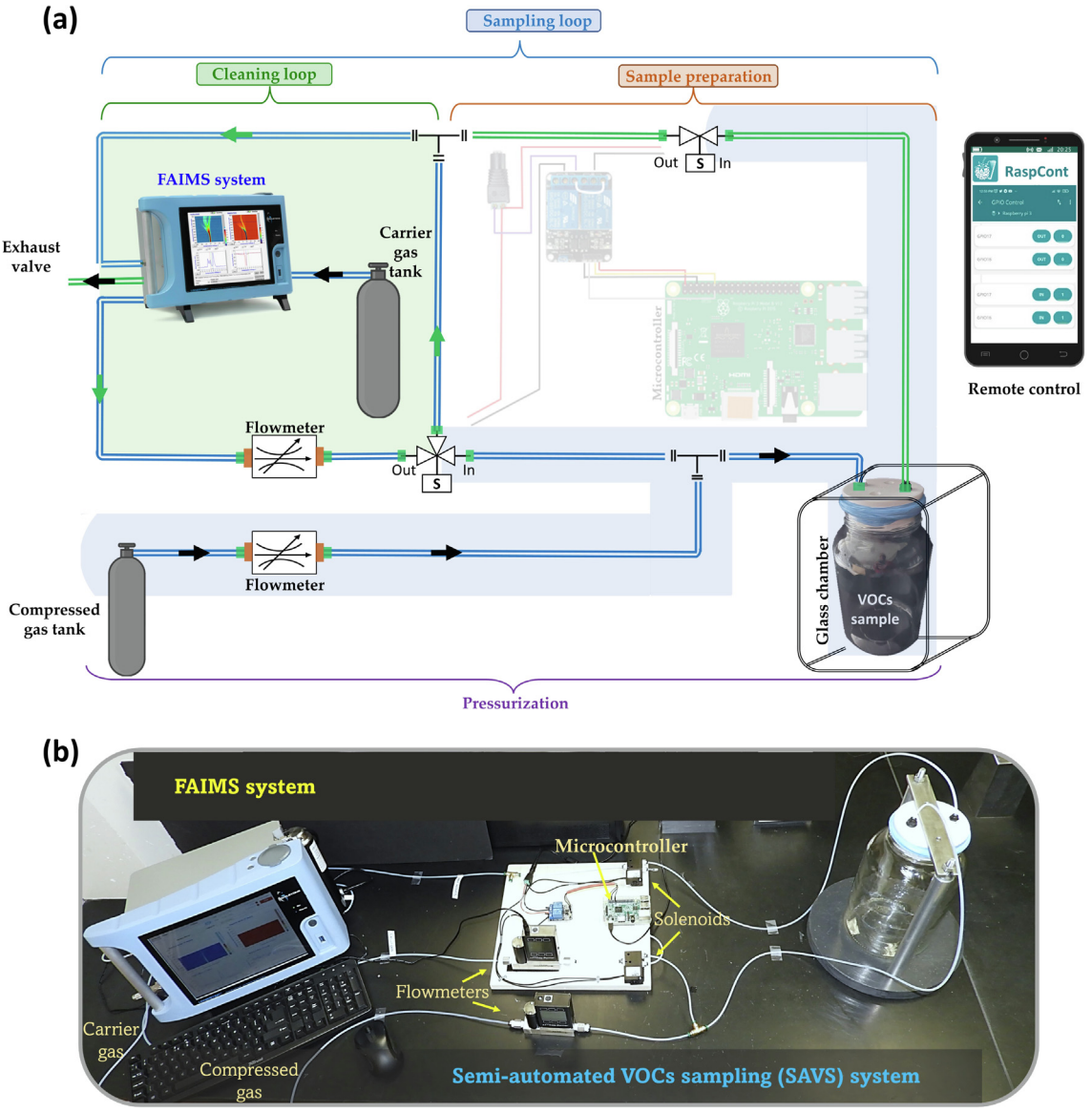


Fig. 3. (a) Schematic functions of SAVS system (FAIMS system cleaning is highlighted with green background and sample pressurization is highlighted with blue background). (b) SAVS system under sampling operation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Operation instructions

Steps	Instructions
1	Power on the SAVS system components, including Raspberry Pi and Solenoids.
2	Open the mobile application RaspController. Follow instructions as provided in: https://www.gallinaettore.com/android_apps/raspcontroller/raspberrypi_configuration_for_raspcontroller/ to establish a connection with SAVS system. Set the GPIO 17 and 18 associated with solenoids operation, this will display in the mobile app (OUT and IN modes).
3	Power on FAIMS system.
4	Open the tank with carrier gas flow. Set pressure at 250,000 (Pa).

(*continued*)

Steps	Instructions
5	Power on the flowmeter one (Fig. 2b) and set the desired gas flow value in liters per minute. This parameter is interrelated with the FAIMS system pre-optimized pressure setting. Therefore, it is recommended to adjust the flow rate and pressure using a flowmeter and FAIMS exhaust valve regulator.
6	The solenoids valves should be closed (OUT mode) using the mobile app (Fig. 3, highlighted in green background color) to purge/clean the FAIMS system.
7	During the above process, the VOC sample can be added to the CHSA by removing the sample chamber stopper. Once the sample is added and stopper replaced, the second tank is used to pressurize the sample (Fig. 3, highlighted in blue). The pressurization takes about 60 s at a flow rate of 3 L/min based on the chamber volume. (Note: Do not exceed the pressure. Check the glass chamber volume and pressure specification)
8	After the FAIMS system is cleaned (based on signal profile of the carrier gas and ion current at 80 % of DF, which should be less than 0.2), VOCs sampling can begin. The solenoids are opened (IN mode) using the mobile app, and immediately the VOCs profiles can be recorded using the FAIMS system.
9	Repeat steps 6, 7, and 8 for collecting and analyzing each sample.
10	Finally, the carrier gas flow is stopped, the SAVS system is turned off using the mobile app, power supplies are disconnected, and the FAIMS system is turned off.

Validation and results

Data collection

The experiment using VOC (acetone) was performed with the CHSA and SAVS system integrated with the FAIMS system (Owlstone, Lonestar, UK) to demonstrate the SAVS system integration functionality. For CHSA and SAVS systems, six scans were collected using an acetone concentration of 690 ppm and 345 ppm, respectively (Fig. 4a, b). The desired acetone concentrations were produced in a volume of 3.81 L (includes glass chamber and tube connectors volume of 3.80 and 0.01 L, respectively) using the liquid injection method (4 and 8 μL for 380 ppm and 690 ppm, respectively) and equation reported in Nakamoto et al. [27]. Data collection was performed with and without the SAVS system for both sampling methods. FAIMS system was operated at 1.6 L/min and 60000 Pa.

Data analysis

Preliminary data exploration indicated that scan #1 displayed large ion current variation using both CHSA and SAVS system. For this reason, scan #2 to scan #6 were used for variability analysis. Review DF Matrix File Offline software (Owlstone ltd, Cambridge) was used to convert dispersion field matrix files (.dfm) to txt files (.txt). A Python (Version 3.8.0) script [19] was used to read text files, extract FAIMS data that incorporates compensation voltage (V), dispersion field (%), and ion current (A.U) in Excel prior to further analysis. Seaborn.heatmap package was used to display the acetone profiles as a

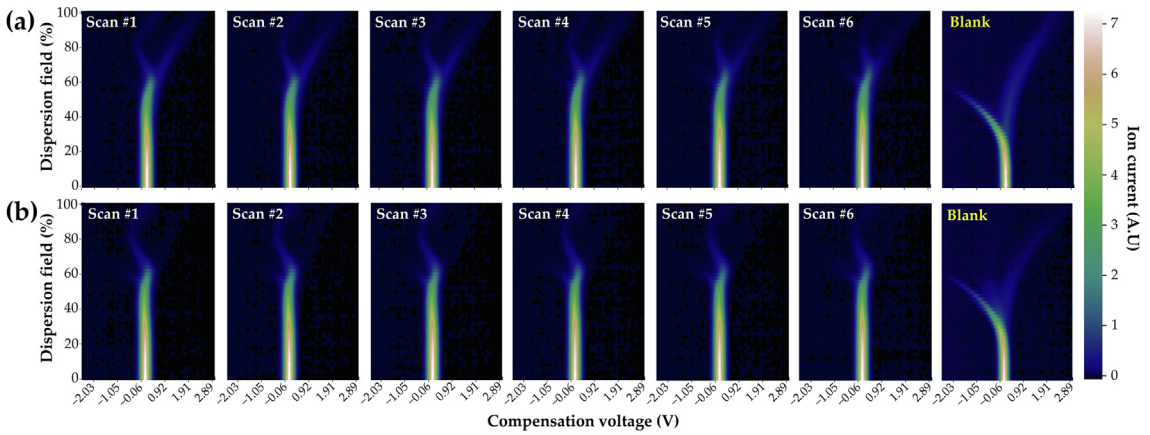


Fig. 4. Acetone profiles acquired using FAIMS system. (a) CHSA. (b) SAVS system. The blank signal represented the ion current profile of the carrier gas.

color-encoded matrix. Finally, the data at 74 % dispersion field from all the scans were analyzed across compensation voltage, which included line plot visualization, standard error calculation, t-test analysis, and standard error visualization. This visualization and analysis were performed on Excel (Microsoft Excel®, Version 2206).

Results and discussion

The pressurization improvement provided by the SAVS system allowed sampling of headspace acetone profiles within five seconds during sampling (time required to reach 1.6 L/min at 60000 Pa), while the CHSA took ~ 90 *sec*. This optimization would also avoid potential volatile sample losses. The acetone evaluated concertation displayed two ion peaks at 690 ppm and one peak at 345 ppm (Fig. 4a, b). These peaks could be a result of intermediate ion acetone reactions that form monomer and dimer molecules as determined from the FAIMS spectra. Fig. 4a and b displays the ion currents intensities across compensation voltages and dispersion fields from the FAIMS spectra of acetone using CHSA and SAVS system.

As a result of ion current variability, three prominent regions were detected in spectra using the CHSA (Fig. 5a) and two prominent regions in the spectra with the SAVS system (Fig. 5b). The standard error associated with these regions indicated a large variation in CHSA compared to the SAVS system (Fig. 5c). This variation was confirmed with *t*-test analysis that provided a significant difference in both sampling methods (P-value: 0.03). In addition, the maximum peak values across scans showed a high coefficient of variation for spectra acquired using the CHSA (16.2 %) than the SAVS system (4.5 %).

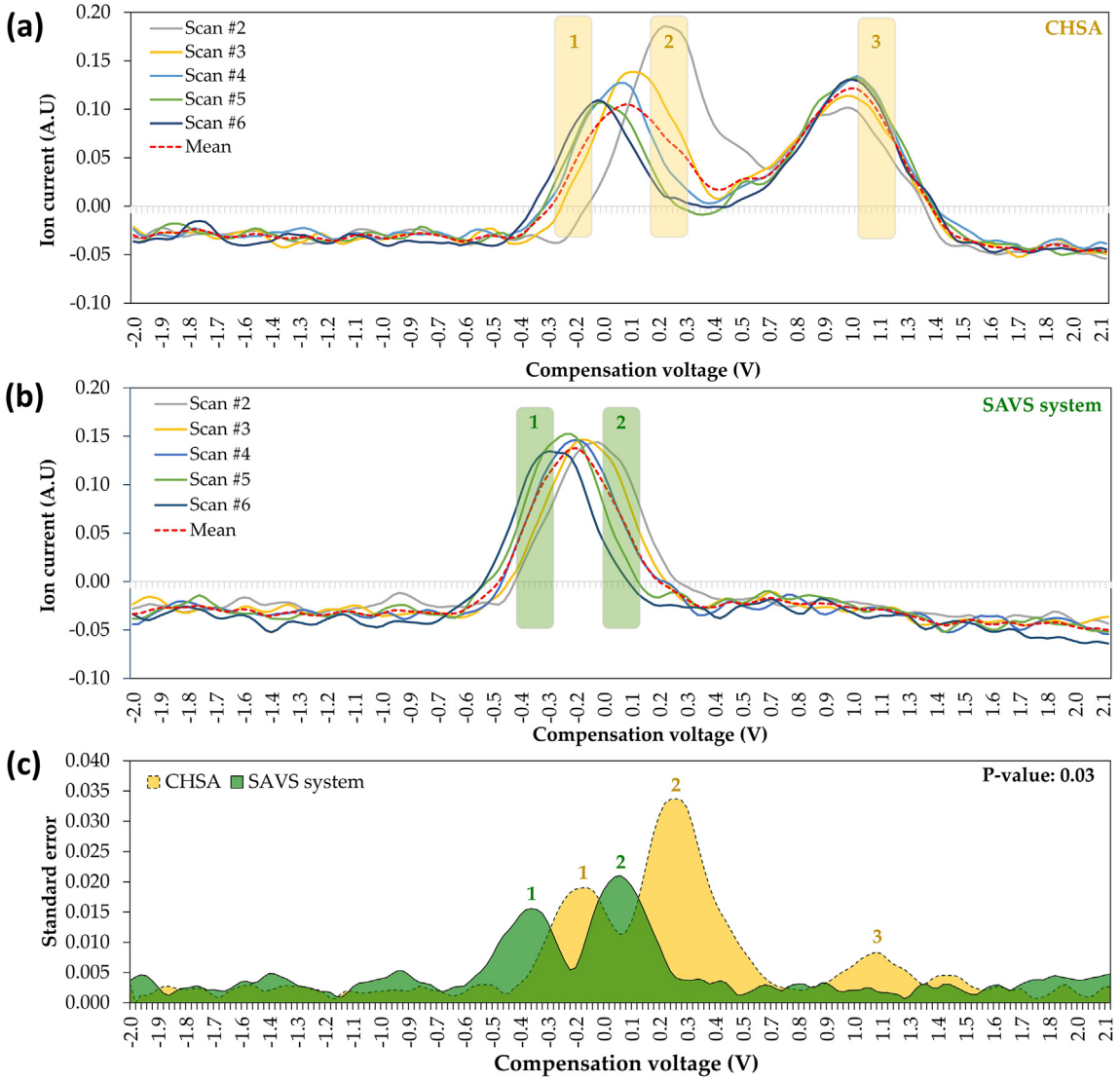


Fig. 5. Ion current variability across compensation voltage (74% dispersion field). Acetone sampled using (a) the CHSA and (b) the SAVS system. (c) Standard error comparison in FAIMS spectral profiles between the CHSA and SAVS sampling systems.

The FAIMS spectral results suggest that the SAVS system provides a more stable acetone profile than sampling with the manual CHSA. Besides, the SAVS system allowed separated cleaning and sampling functions, as well as sample preparation and pressurization. The separation of these functions was critical to reducing acetone losses during tubing connections and sampling. Despite the SAVS system capabilities demonstrated in this study, the system can be further improved easily. The improvements can be: (i) inclusion of multiple CHSA units for continuous sampling from different sample chambers, (ii) integrating the flowmeters with a microcontroller for remote operation, and (iii) automating the control of flowmeter and solenoid using a programmed software. Moreover, the SAVS system can be further miniaturized. Such automation is essential to characterize VOCs for multiple applications in agriculture such as plant and soil pathogen detection, evaluation of crop responses, chemical profile characterization, etc.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Milton Valencia-Ortiz received his B.S. in Agronomic engineer in 2010 and his M.S. Agricultural Sciences in 2014 from Universidad Nacional de Colombia. He had worked at the Alliance of Bioversity International and CIAT, Cali, Colombia in the Biotechnology Research Unit from 2014 to 2021. Currently, he is a Ph.D. candidate in Professor Sindhuja Sankaran's phenomics lab at Washington State University (Department of Biological Systems Engineering). His current research focuses on plant phenotyping using proximal and remote sensing technologies on breeding trials.

Sindhuja Sankaran is based at Washington State University in Pullman, Washington, her work focuses on advanced sensor technologies that detect and measure phenotypes—utilizing opto-electronic, biological, and chemical sensors for non-invasive, rapid and continuous monitoring of crop responses to environmental stress, helping create a faster and better understanding of how our food crops react to a changing environment. Her work on advanced sensor technologies supports plant breeding, crop plant research, and precision agriculture applications. She holds a doctorate in Agricultural and Biosystems Engineering, master's degrees in Environmental Engineering and Environmental Science, and a bachelor's degree in Zoology.