

PAPER • OPEN ACCESS

## A low-cost internal standard loader for solid-phase sorbing tools

To cite this article: F M Vivaldi *et al* 2023 *J. Breath Res.* **17** 046008

View the [article online](#) for updates and enhancements.

You may also like

- [One-Compartment Hydrogen Peroxide-Photofuel Cell Using TiO<sub>2</sub> Photoanode and Prussian Blue Cathode](#)  
Atsunobu Akita, Takumi Masuda, Keigo Fujiwara et al.
- [Study of an unfolding algorithm for D-T neutron energy spectra measurement using a recoil proton method](#)  
Jie Wang, , Xiao-Long Lu et al.
- [Breath Acetone Analysis Using a Porous Glass Sensor Developed By Impregnating with 4-Nitrophenylhydrazine](#)  
Kodai Ito, Asuya Suzuki and Yasuko Yamada Maruo



**Breath Biopsy Conference**

Join the conference to explore the latest challenges and advances in breath research

31 OCT - 01 NOV  
ONLINE

Register now for free!

BREATH BIOPSY




The banner features a dark background with orange and white text. On the right, there is a photograph of a diverse group of people at a conference, with a 'BREATH BIOPSY' logo in the top right corner.



## PAPER

## A low-cost internal standard loader for solid-phase sorbing tools

## OPEN ACCESS

F M Vivaldi<sup>1,\*</sup> , S Reale<sup>1</sup>, S Ghimenti, D Biagini, A Lenzi, T Lomonaco<sup>\*</sup>  and F Di Francesco 

Department of Chemistry and Industrial Chemistry, University of Pisa, via Giuseppe Moruzzi 13, 56124 Pisa, Italy

<sup>1</sup> These authors equally contributed to this work.

\* Authors to whom any correspondence should be addressed.

E-mail: [federicomaria.vivaldi@phd.unipi.it](mailto:federicomaria.vivaldi@phd.unipi.it) and [tommaso.lomonaco@unipi.it](mailto:tommaso.lomonaco@unipi.it)**Keywords:** solid phase extraction, needle trap microextraction, internal standard, quality control, breath analysisSupplementary material for this article is available [online](#)

## RECEIVED

20 March 2023

## REVISED

2 August 2023

## ACCEPTED FOR PUBLICATION

11 August 2023

## PUBLISHED

29 August 2023

Original content from this work may be used under the terms of the [Creative Commons Attribution 4.0 licence](#).

Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

**Abstract**

Solid-phase sorption is widely used for the analysis of gaseous specimens as it allows at the same time to preconcentrate target analytes and store samples for relatively long periods. The addition of internal standards (ISs) in the analytical workflow can greatly reduce the variability of the analyses and improve the reliability of the protocols. In this work, we describe the development and testing of a portable system for the reliable production of gaseous mixture of <sup>8</sup>D-Toluene in a 1L Silonite canister as well as its reproducible loading into solid-phase sorbing tools as ISs. The portable system was tested using needle trap microextraction, solid-phase extraction, and thin-film microextraction techniques commonly employed for the analysis of gaseous samples. Even though our specific interest is in breath analysis, the system can also be used for the collection of any kind of gaseous specimen. A microcontroller allows the fine control of the sampling flow by a digital mass flow controller. Flow rate and sample volume could be set either through a rotary encoder mounted onto the control board or through a dedicated android app. The variability of the airflow is in the range 5–200 ml min<sup>-1</sup> and it is lower than 1%, whereas the variability of the IS (<sup>8</sup>D-Toluene) concentration dispensed over time by the loader measured by selected-ion flow-tube mass spectrometry (MS) is <3%. This combination resulted in intra- and inter-day precision of the amount loaded in the sorbent tools lower than 15%. No carry-over was detected in the loader after the delivery of the <sup>8</sup>D-Toluene measured by gas chromatography–MS. The <sup>8</sup>D-Toluene concentration in the canister was stable for up to three weeks at room temperature.

**1. Introduction**

Solid-phase sorption, including both extraction and microextraction techniques, is widely used in analytical chemistry for the analysis of complex mixtures. It is particularly useful for the analysis of gaseous samples, as it allows at the same time to preconcentrate target analytes and store samples for relatively long periods (e.g. up to months) [1]. Among sampling tools, sorbent tubes analysis (STA), solid-phase microextraction (SPME) fibers, and needle trap microextraction (NTME) tools are particularly interesting [2–6]. All these sampling tools present pros and cons depending on the type of analysis and application. STA allow both active and passive

sampling. In the first case, a pump is used to collect the specimen, in the second case diffusion is exploited. For a reliable quantification of the target analytes, the STA approach requires a perfect analyte retention during sampling, so breakthrough volume must not be exceeded [7]. Sensitivity of the analytical protocol can be improved by increasing the sample volume as well as avoiding the load of an excessive amount of water in case of humid samples, such as breath [8]. Needle trap devices (NTDs) and SPME fibers are common alternatives to STA for the determination of volatile organic compounds (VOCs) in breath samples [9] and for the analysis of head space (HS) of biological specimens [10], respectively. NTDs are widely used for breath analysis since they allow

to achieve similar analytical performances (e.g. limit of detection) of STA with one tenth of sample. Such limited volume makes it easy to avoid the use of sampling bags and reduces the sampling time. A main strength point of NTDs is the limited collection of water during sampling as well as the possibility of performing thermal desorption manually or automatically in the gas chromatography (GC) inlet, without a dedicated unit [11]. Thanks to the small size, lightness, and strength of the needle, the NTME approach is easily transportable and allows in-situ sampling. Literature reports the application of NTME technique both in clinical [12] and environmental [13] studies. Despite being of very simple use, SPME fibers have a small amount of adsorbent material on the fiber, which limits the extraction capability of the sorbent phase, and are quite fragile [14]. Sorptive-based extraction methods such as thin film microextraction (TFME) [15] and stir-bar sorptive extraction (SBSE) offer an alternative approach [16] that overcomes the limited amount of sorbent phase in contact with the sample and poor extraction capacity typical of SPME fibers. The SBSE is an evolution of the SPME method in which the stationary phase volume is increased (9 vs. 0.6  $\mu\text{l}$ ), thus significantly improving sensitivity and extraction capacity [17]. SBSE is more robust than the fiber but the phase mainly used is polydimethylsiloxane (PDMS), characterized by a low affinity towards more volatile and polar compounds. Due to the larger stationary phase volume, the equilibration time is longer. The TFME technique overcomes both the limitation of SBSE (membranes with different stationary phases are available) and SPME fiber (larger stationary phase volume in contact with the sample), thus improving the extraction capacity. These devices are robust, reusable, and flexible. A disadvantage of using a phase with a larger volume is the consequent increase of background and the tendency to soil the instrument, which makes a frequent cleaning of the instrument necessary [18]. TFME, SPME and SBSE techniques have been applied in many fields, including industrial, food, environmental and medical. Whatever the technique chosen for the collection of the gaseous sample, the addition of a precise amount of an internal standard (IS) into the analytical workflow improves the precision and accuracy of methods by correcting for uncontrolled sample losses during sample preparation and storage, as well as for any potential variations related to the sorbing efficiency of the sorbent materials. For example, the loading of an IS into the stationary phase has been proposed to improve the reliability of NTME-based protocols when working with gaseous samples characterized by different humidity values (i.e. breath and ambient air) [19]. Despite the importance of ISs in the analytical measurements, its improper use does not reduce the method variability, so well-defined procedures to load reproducible amounts of IS into sorbent phase are recommended.

In this work, we propose a protocol for the preparation of a gaseous standard in a Silonite canister to be used in combination with a lab-made prototype for the reliable loading of specific volumes of the mixture into a sorbent phase. As proof of concept, we tested  $^8\text{D}$ -toluene as IS and NTME, STA, and TFME as representative sample collection tools. The proposed protocol, combined with the automatization of the IS addition into the sorbent phase, represents a step forward towards reduction of method variability for a reliable breath analysis.

## 2. Materials and methods

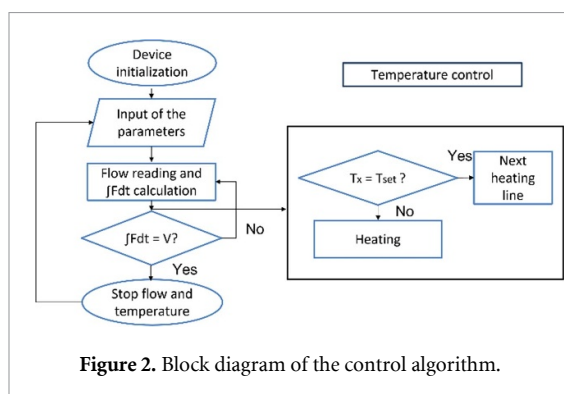
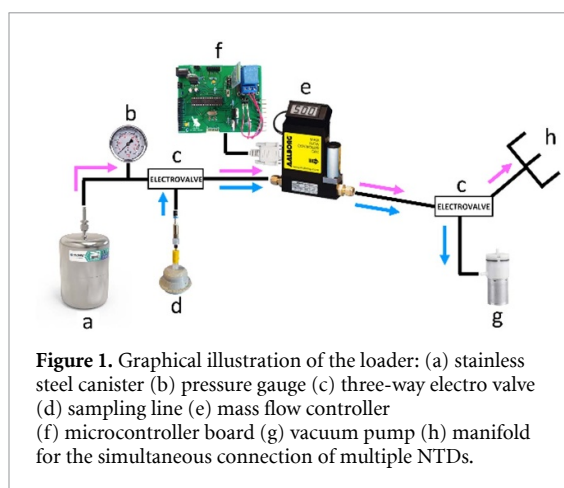
### 2.1. Reagents and materials

Methanol (MeOH, purity 99.99%) was purchased from Merck (Italy), whereas  $^8\text{D}$ -Toluene (purity of 99.8%) was purchased from ARMAR Chemicals (Switzerland). A 1L Silonite canister (MiniCans<sup>TM</sup>) was purchased from Entech Instruments (Italy). The digital mass flow controller (MFC, GFC17A-BAL6-A0) was from Aalborg Digital (USA). The protective case (Peli<sup>TM</sup> Protector 1400EU) was purchased from Peli (Spain). The electronic components were purchased from RS components (Italy). Polytetrafluoroethylene (PTFE) tubes (inner diameter of 1 mm) were purchased from Supelco (Italy). Single phase NTDs (23-gauge, 6 cm long) were packed with 3 cm of Tenax GR 60/80 mesh (70% Tenax TA, i.e. 2,6-diphenyl-p-phenylene oxide, and 30% graphite) and were purchased from PAS-Technology (Germany). Stainless-steel sorbent tubes (O.D. 6.4 mm, I.D. 5 mm, 89 mm length) packed with 250 mg of 60/80 mesh Tenax GR phase were purchased from Markes International (UK). TFME (40  $\times$  4.85  $\times$  0.04 mm) in PDMS/Carboxen were purchased from Markes International (UK).

### 2.2. Device assembly

Figure 1 shows the connections among the loader components.

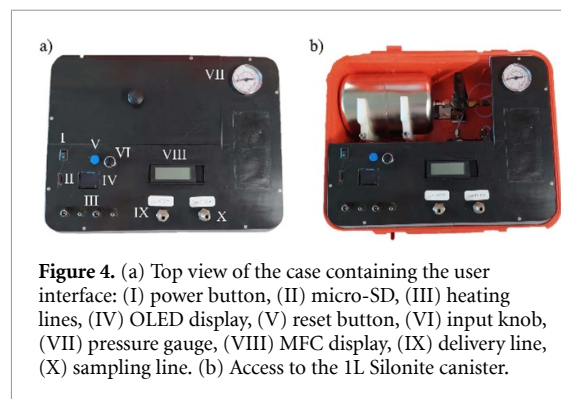
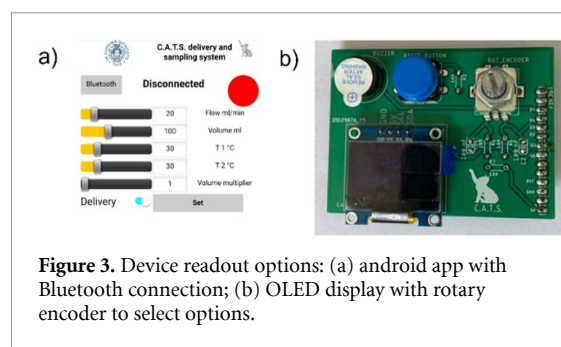
The system is versatile since it allows to divert the flow rate thanks to the action of two electrovalves (Honeywell, USA). In *sampling mode*, aliquots of gaseous samples (e.g. ambient air or breath collected with bags) may be transferred into sorbent tools, whereas in *loader mode*, the system dispenses known volumes of a gaseous standard solution stored in the Silonite canister. Blue arrows indicate the flow rate when the system operates in *sampling mode*, whereas the pink arrows indicate the flow when the system operates in *loader mode*. The canister was connected to a pressure gauge for reading its residual pressure and then to the system through a series of PTFE tubings. The lab-made prototype was equipped with two heating lines that could be used to set the temperature of tubing carrying the sample at 40  $^{\circ}\text{C}$  to avoid condensation. The electronic board controlling the MFC, the heating system, and the user interface were



developed using an ATMEGA328PU microcontroller and was powered from a single power supply (12 V). Additional outputs were also available for Bluetooth and serial connections, and digital I/O pins to handle the sample pump and the physical user control board. The temperature of each line was measured by an NTC2.2K3359I (Farnell, Italy) thermistor in contact with the heating resistance. Upon reaching the target temperature (e.g. 40 °C) due to the Joule effect, an IREZ44N MOSFET (RS Components, Italy) switched off the electrical current. Figure S1 shows the printed board with the soldered components.

The algorithm, written in Arduino code using visual studio as code editor, is summarized in the block diagram shown in figure 2. The system readout was achieved through an android app using a Bluetooth connection (figure 3(a)) or a modular board with an organic light-emitting diode (OLED) display, whereas either the app or a rotary encoder could be used to select options (figure 3(b)).

All the electronic and pneumatic components were arranged in a case (figure 4): system components not intended to be touched from the user such as connections, MFC, solenoid valves, air pump, and electronic board were protected in a lower level, whereas the user interface composed of power button (I), micro-SD card for data logging (II), outputs for the cables of the two heating lines (III), display (IV), blue reset button (V), knob for user input (i.e., flow



volume and temperature) (VI), pressure gauge (VII), MFC display showing the measured flow (VIII), and two lines for connection with the system for loading the IS (IX) and for sampling (X) were located in the upper level (figure 4(a)). The easy access to the canister was guaranteed by the presence of a removable cover as shown in figure 4(b).

### 2.3. Preparation of the <sup>8</sup>D-Toluene standard solutions

A stock liquid standard solution of <sup>8</sup>D-Toluene was prepared by diluting 300 times pure <sup>8</sup>D-Toluene in MeOH, then aliquots were stored in 1 ml amber vials at 4 °C up to one month. A stock gaseous standard mixture of <sup>8</sup>D-Toluene was prepared by manually injecting 10 μl of methanol solution into a 1L Silonite canister at low pressure (0.6 psi) equipped with a two-way valve and an IN-Stopper (B.Braun, Italy). After the injection, the valve was immediately closed and the canister was heated at 40 °C for 30 min to equilibrate the gaseous solution. Then, the canister was filled with pure nitrogen up to 37 psi using a helium diffusion system preparation station (Entech Instruments, Italy) and was ready to be used. Additionally, the canister could be stored at room temperature up to 3 weeks. After this procedure, the canister contained <sup>8</sup>D-Toluene at 3.2 ppmV. Before preparing a gaseous standard, the canister was (a) vacuum cleaned at a pressure of 0.6 psi, (b) rinsed three times with MeOH (3 × 50 ml), (c) placed in an oven at 60 °C to remove methanol leftover, and then (d) kept under vacuum overnight at 60 °C.



## 2.4. Sample analysis

### 2.4.1. Method 1: NTME analysis

Prior to first use, single-bed NTDs were conditioned for 15 h at 300 °C with 14.5 psi of N<sub>2</sub> using a commercial heater block (PAS-Technology, Germany). Subsequently, needle traps were sealed with Teflon caps and stored in glass containers at room conditions. Just before use, NTDs were further conditioned for one hour at 300 °C using the same heater. Needle traps were automatically desorbed for 20 s at 300 °C at a pressure of 16.24 psi using a Concept auto-sampler (PAS-Technology, Germany) and an Agilent 5190–7045 injector (internal volume of the liner equal to 35 μl). We worked in split mode with a ratio of 1:2. During the desorption, a septum purge of 3 ml min<sup>-1</sup>, a column flow of 1 ml min<sup>-1</sup> and a split flow of 5 ml min<sup>-1</sup> were applied for a total flow of 9 ml min<sup>-1</sup>. Analytes were analyzed by a 7890B GC (Agilent Technologies, USA) coupled to a 7010 triple quadrupole GC/mass spectrometry (MS) (Agilent Technologies, USA) equipped with an electron impact ion source operating at 70 eV. The chromatographic separation was performed using a DB-624 capillary column (60 m × 0.25 mm, 1.4 μm film thickness) purchased from Agilent Technologies (USA) of medium polarity, composed of 6% cyanopropyl/phenyl and 94% polydimethylsiloxane (PDMS). He 5.5 HP was used as the carrier gas at a constant flow rate of 1 ml min<sup>-1</sup>. The temperature program was the following: 35 °C for 15 min, 4 °C min<sup>-1</sup> up to 130 °C maintained for 2 min, 10 °C min<sup>-1</sup> up to 250 °C maintained for 1 min. The mass spectrometer operated in full scan mode (range of masses studied from m/z 35 to m/z 500) using a scan time of 300 ms. Temperatures of the transfer line, ion source, and quadrupoles were set at 260 °C, 250 °C and 150 °C respectively. Helium and nitrogen were used as the quench gas (flow of 4 ml min<sup>-1</sup>) and the collision gas (1.5 ml min<sup>-1</sup>), respectively. A 5 min solvent delay was set to protect the filament from water vapor released during the thermal desorption process. Chromatographic data were acquired using MassHunter software (v. B.07.00, Agilent Technologies, USA).

### 2.4.2. Method 2: TFME and STA

Prior to their first use, TFME membranes were conditioned for 60 min at 250 °C under a 70 ml min<sup>-1</sup> flow of N<sub>2</sub> in a thermal conditioner (TC20, Markes, UK). Subsequently, the membranes were stored in empty stainless-steel tubes at room conditions sealed with Swagelok caps. Prior to sampling, TFME were conditioned for 30 min at 280 °C. In the same way, sorbent tubes were conditioned for the first time for 120 min at 300 °C under a 70 ml min<sup>-1</sup> flow of N<sub>2</sub> in TC20. After use, sorbent tubes were immediately conditioned for 60 min at 300 °C. Samples were analyzed by a TD unit (TD-100, Markes International, UK) coupled with a GC/MS detector (Models 7890B/7010,

Agilent Technologies, USA). During the primary desorption stage, analytes were desorbed in spitless mode at 280 °C (TFME membranes) or 300 °C (STA) for 10 min by applying a helium flow rate of 50 ml min<sup>-1</sup> and then refocused onto a cryogenic-trap at 5 °C. Secondary desorption was carried out in split mode (split ratio 1:10) by a rapid heating of the cold trap at 300 °C, which was kept under these conditions for 20 min. During all TD steps, the flow path temperature was set at 140 °C. The chromatographic separation was performed using a non-polar phenyl arylene polymer capillary column (DB-5 ms, 60 m × 0.25 mm, 1 μm film thickness, Agilent Technologies, USA). He 5.5 HP was used as the carrier gas at a constant flow rate of 0.8 ml min<sup>-1</sup>. The temperature program was: 30 °C for 13 min, 4 °C min<sup>-1</sup> up to 130 °C maintained for 3 min, 10 °C min<sup>-1</sup> up to 250 °C maintained for 7 min. The mass spectrometer worked under the same conditions described for the NTME analyses (section 2.4.1).

## 2.5. Test of the prototype

### 2.5.1. Monitoring air flow through the device in both sampling and loader mode

The accuracy of the flow rate was assessed by measuring the flow rate with a digital bubble flow meter (Mini-Buck Flow Calibrator, Supelco, flow range 1–6000 ml min<sup>-1</sup>) connected to the system. Preliminary tests using the *loader mode* were carried out with a stable N<sub>2</sub> flow, in the range 5–200 ml min<sup>-1</sup>, provided from lab supply (E1). Experiments were then replicated using the Silonite canister filled with N<sub>2</sub> up to 37 psi as gas source (E2). Experiment E3 simulated a real case scenario in which aliquots of the gaseous sample were loaded into NTDs and STA. Flow rates through the NTDs (5, 10, 15, 20 ml min<sup>-1</sup>) were selected to maximize the interaction between the target analyte and the sorbent phase, as discussed elsewhere [13, 20]. The flow rate in *sampling mode* was tested connecting either sorbent tubes or NTDs (E4). During the experiments the serial communication was used to monitor the readout of the system.

### 2.5.2. Monitoring of temperature values of the heating lines

Condensation is an usual problem when warm breath samples are collected from the oral cavity at temperatures ranging between 31.4 °C and 35.4 °C [21]. To avoid the potential partitioning of water-soluble analytes between the water film and the gaseous sample, all materials in contact with breath should be heated at a minimum temperature of 40 °C. Our prototype can also be used to continuously transfer gaseous samples into NTDs or aliquots of breath samples, previously collected with polymeric bags, into sorbent tubes. Thus, we decided to implement two heating lines in the system to warm up all materials during sampling. Experiments were carried out to evaluate the robustness of the temperature to fluctuations

when the device was used in sampling mode at 5, 10, 15, 20, 50, 100, 150, and 200 ml min<sup>-1</sup>.

### 2.5.3. Blank samples and evaluation of the carry-over effect

For routine analysis, system components such as Silonite canister, tubing and fittings should be as clean as possible (limited carry-over effect). Blank samples were carried out by analyzing 50 ml of pure N<sub>2</sub> flown through the prototype and collected with all the extraction tools under test. After cleaning, blank samples collected from the canister were tested. In the same way, the effectiveness of the cleaning procedure was evaluated by analyzing blank samples collected after the first preparation of the stock gaseous standard solution. Carry-over was calculated as the ratio between the area of the quantifier <sup>8</sup>D-Toluene ion (98) before and after cleaning.

### 2.5.4. Reproducibility of the gaseous standard preparation and stability of 8D-Toluene over time

The reproducibility of the gaseous standard preparation was estimated by comparing the area of the chromatographic peak of the quantifier <sup>8</sup>D-Toluene ion (98) using all the extraction tools under test over three different days. For each type of device, analyses were performed in triplicate.

The stability of <sup>8</sup>D-Toluene signal in real time during a complete emptying of the canister was evaluated by means of a selected ion flow tube (SIFT) MS (Voice 200 Ultra, Syft Technologies, New Zealand). A standard gaseous solution was prepared as described in 2.3. The canister was then connected to a three-way union allowing the SIFT-MS to collect air at 25 ml min<sup>-1</sup> while emptying the canister at 50 ml min<sup>-1</sup> through a heated (120 °C) outlet. Figure S2 shows the experimental setup for SIFT-MS analysis. Reagent ions (i.e., H<sub>3</sub>O<sup>+</sup> and NO<sup>+</sup>) were formed in a microwave discharge through moist air at a pressure of 0.5 mbar to react with <sup>8</sup>D-Toluene and produce m/z (NO<sup>+</sup>) = 100 and m/z (H<sub>3</sub>O<sup>+</sup>) = 101 product ions, according to the mechanisms reported elsewhere [22]. They are selected according to their mass-to-charge ratio by a quadrupole mass filter and then injected into the flow tube to react with <sup>8</sup>D-Toluene and produce m/z (NO<sup>+</sup>) = 100 and m/z (H<sub>3</sub>O<sup>+</sup>) = 101 ions. The dwell time for each mass was 300 ms. LabSyft Pro Method Editor software (SYFT Technologies) was used for data acquisition and analysis. The SIFT-MS instrument was calibrated using a multiple component certified Syft Calibration gas standard (Syft Technologies, New Zealand). The stability at room conditions (25 °C) of <sup>8</sup>D-Toluene was evaluated by analyzing three aliquots of the stock solution every week up to 1 month. The concentration at *t* = 0 h was used as the reference value. The sample's stability was evaluated by means of the analysis of variance (ANOVA) at a confidence level of 95%.

## 2.6. Application of the prototype in real case scenario

As a proof of concept, our prototype was used for loading a known amount (0.12 μg) of <sup>8</sup>D-Toluene into NTDs, sorbent tubes and TFME tools. In two months of routine analysis, we successfully analyzed 70 NTDs, 70 STA, and 40 TFME membranes. Data were treated using the 'qcc' R package.

## 3. Results

### 3.1. Test of the prototype

The prototype is extremely flexible and works in two modalities: (i) *sampling mode* and (ii) *loader mode*. The first allows collecting a volume of gaseous sample (e.g. room air or breath) into a sorbent device, whereas the second allows the load of known IS volumes in the same device. In both cases, the electronic board monitors and controls in real-time the flow through the two lines thanks to an MFC. A manifold permits the connection and the simultaneous loading of IS into multiple devices.

### 3.2. Precision and accuracy of the flow rate and temperature values

Table S1 reports the flow rate values measured during 20 min tests. The prototype demonstrated a high precision and a relative standard deviation (RSD) lower than 2% (with the exception of one flow rate of the E4 experiment, whose RSD was 3.60%). In addition, precision was independent from the gas supply, confirming the canister as a portable IS source. The accuracy of the nominal flow set by the micro controller and measured through the serial communication was evaluated using a digital bubble flow meter (figure S3). A maximum difference of 10% between nominal flow and flow measured from the bubble flow meter was observed.

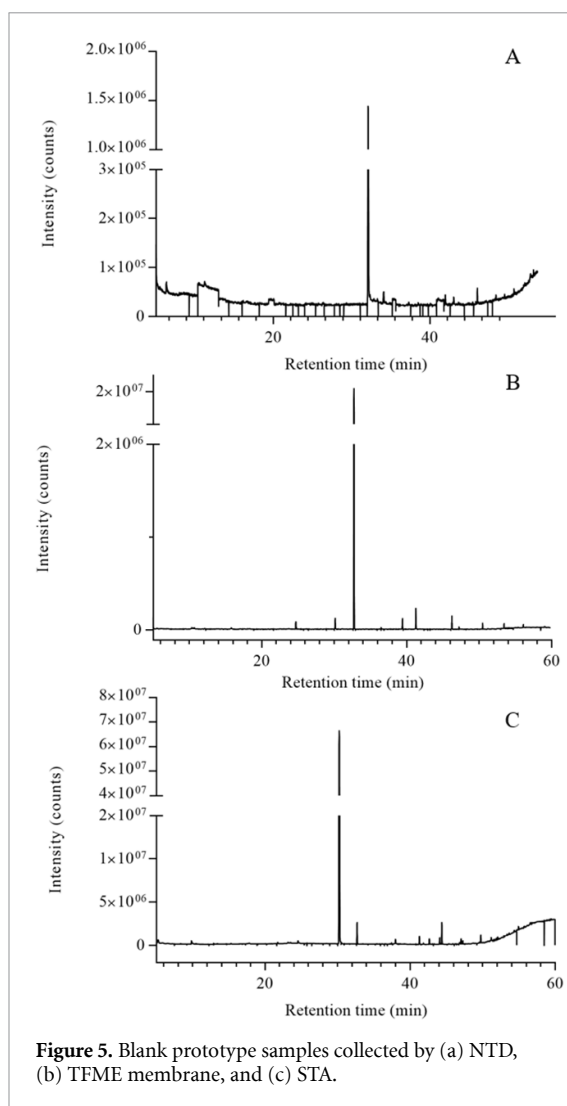
To verify the stability over time of the two heating line temperatures, tests were carried out by setting a temperature of 40 °C and varying the gas flow values in the range of 5–200 ml min<sup>-1</sup> (table S2). Temperature fluctuations were within 10% and the maximum difference from the set value was about 8%.

### 3.3. Background signal and carry-over effect

Figure 5 shows typical total ions current chromatograms of blank samples obtained with all the extraction tools. In all cases, the prototype showed a negligible VOCs background, thus confirming the low emission rate of the materials. The carry-over of IS was estimated at 2%, thus confirming the effectiveness of the cleaning procedure of the system.

### 3.4. Reproducibility of the gaseous standard preparation and stability of 8D-Toluene over time

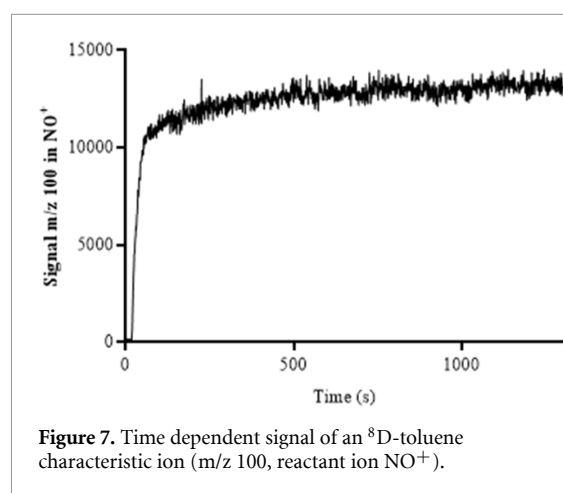
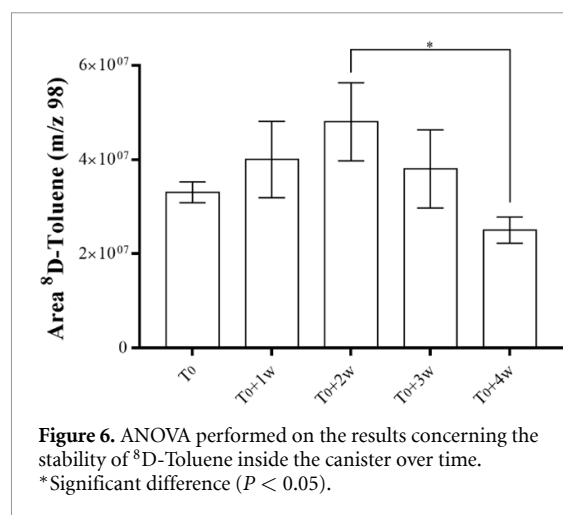
Intra-day variability, expressed as the RSD of the areas of <sup>8</sup>D-Toluene peaks resulting from desorption of



NTDs ( $n = 3$ ), was lower than 10%, which agrees with the inter-needle variability observed in previous studies [12]. Inter-day reproducibility was evaluated by analyzing gaseous standards prepared on three different days by the same operator. Areas of  $^8\text{D}$ -Toluene measured during the inter-day experiments are compared in table S3 and figure S4(a).

ANOVA test showed the presence of significant differences between results obtained in the second and the third day, even if the overall variability was about 15%, which is an acceptable value considering the complexity of breath samples. To investigate the origin of this variability, we evaluated the operator reproducibility in collecting aliquots of  $^8\text{D}$ -Toluene liquid standard solution. Quintuplicate measurements demonstrated that the addition of the  $10\ \mu\text{l}$  in the canister justified about one third ( $\text{RSD} = 4\%$ ) of the overall variability.

The inter-operator variability was assessed by an experiment in which three operators independently prepared a gaseous standard solution. An ANOVA test excluded significant differences among the operators and confirmed the reliability of the procedure (figure

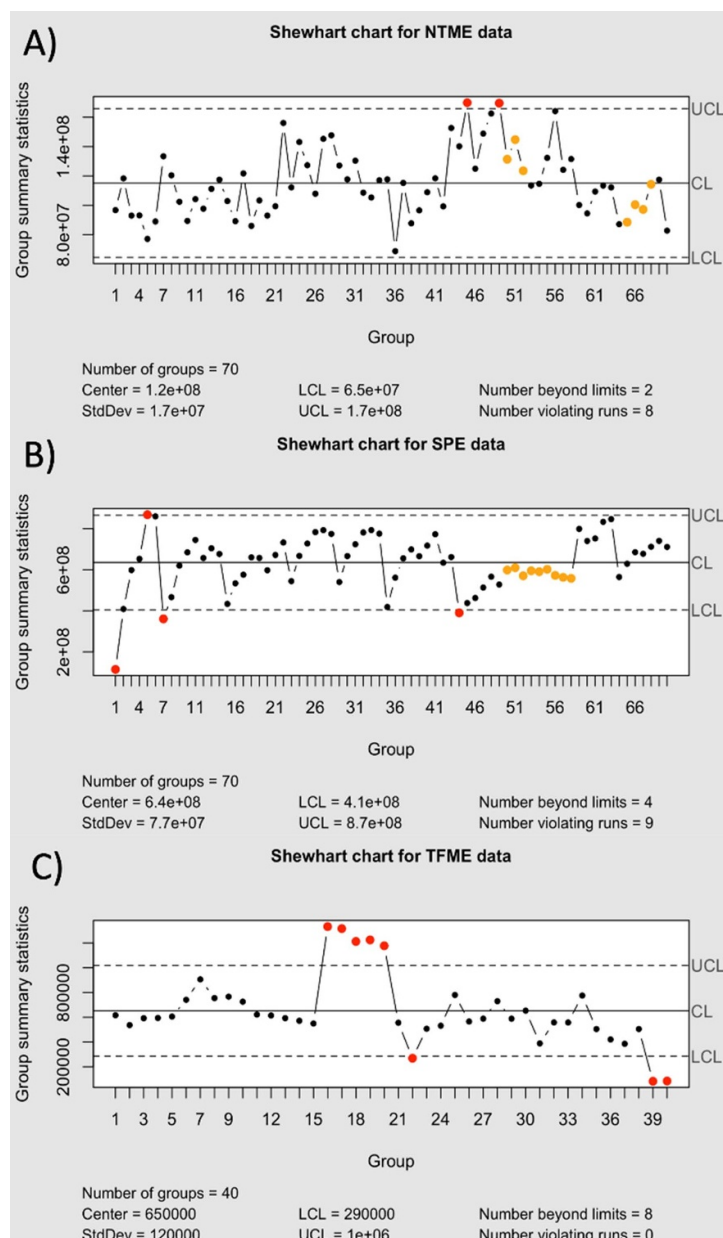


S4(b) and table S4). The RSD calculated from all data was 8%.

Finally, the stability of the IS standard gaseous solution was evaluated over a time span of 1 month. Analyses were repeated weekly by loading three NTDs. Table S5 shows the experimental conditions (temperature, relative humidity, and residual pressure of the canister) at each test. ANOVA test (figure 6) highlighted a significant difference between results obtained at  $T0 + 2\text{w}$  and  $T0 + 4\text{w}$ . For this reason, we decided to store stock gaseous IS solutions at room temperature for up to four weeks. The overall variability excluding the  $T0 + 4\text{w}$  datum was 16%, which is comparable with the variability obtained when assessing reproducibility of measurements.

### 3.5. Delivery of the IS over time

Figure 7 shows the signal over time of one characteristic ion ( $m/z\ 100$ ) produced from  $^8\text{D}$ -toluene upon reaction with  $\text{NO}^+$  in a SIFT-MS instrument. The average signal at plateau after about 200 s was 12 800 counts, with a standard deviation of 500 ( $\text{RSD} = 4\%$ ). These data confirmed the reliable and continuous dispensing of known amounts of  $^8\text{D}$ -Toluene by the prototype during the continuous drop of the residual pressure while emptying the canister. The relatively



**Figure 8.** Shewhart control chart of  $^8\text{D}$ -toluene over a period of 2 months. CL: central line defined as mean. UCL: upper control limit defined as mean +3 sigma, LCL: low control limit defined as mean -3 sigma. Number of  $^8\text{D}$ -Toluene peak areas beyond UCL and LCL limits are highlighted in red, yellow points refer to peak areas violating Nelson rules [24].

long response time, defined as the time necessary to reach the 90% of the signal ( $T_{90} = 150$  s), was mainly a consequence of the dead volume of the SIFT-MS and the relative low flow rate value in its transfer line ( $25 \text{ ml min}^{-1}$ ).

### 3.6. Using the prototype in real case scenarios

The prototype was used daily to deliver known amounts of  $^8\text{D}$ -Toluene into NTDs, STA, and TFME membranes currently employed in our laboratory for breath and air analysis. For each protocol, we plotted a Shewhart control chart assuming that variations of the  $^8\text{D}$ -Toluene signal within the control limits were mainly due to random causes whereas variations outside these limits were due to well-defined

causes [23], such as the aging of the sorbent phase and modification of the desorption efficiency, as well as a change of the instrumental response. As an example, we previously demonstrated the importance of adding labeled ISs directly to NTDs before performing gaseous sampling [19].

Figure 8 shows the Shewhart control chart of  $^8\text{D}$ -Toluene for all the extraction tools over a period of 2 months.

The high precision of the prototype in loading known amounts of IS into the extraction tools allowed it to monitor the analytical performances of the instrumental protocols. The NTME and STA methods were under quality control with an overall method variability close to 10%. Only a few points



(2 for NTME and 4 for STA) exceed the upper control limit (UCL) and lower control limit (LCL) (figures 8(A) and (B)). For both methods, the 8-Nelson rules were sometimes violated, thus suggesting the presence of a specific pattern within the dataset [24]. For example, the presence of nine consecutive data in the STA control chart with data close to the central line (mean) suggests a small shift of the instrumental response. In the case of the TFME method, eight data exceeded the UCL and LCL, thus suggesting a large shift of the method response as confirmed by the change of tuning factor of the mass spectrometer.

## 4. Discussion

In this work, a prototype was developed for the controlled generation of standard gaseous IS mixtures and the reproducible loading of their specific volumes into NTDs, STA, and TFMEs. The loading of an IS makes it possible to compensate for errors due to the loss of analyte during sample treatment and analysis, and to quantify analytes more reliably. The prototype can also work in sampling mode, and in this case set volumes of ambient air or breath are transferred into the sampling device.

The most expensive prototype component is the MFC (800–1000 euros), while the control board can be assembled for less than 50 euros. Two electrovalves permit switching between two gas lines when working in *sampling* or *loading* mode; the accuracy of flow rate was extensively tested by a bubble flow meter, which showed a bias lower than 10%. In order to take this discrepancy into account, we included a corrective factor within the source code. Temperature of two heated lines could be set during sampling of breath or ambient air to prevent the condensation of water. The analytical performances of the prototype were tested with several instrumental techniques in loading known amounts of <sup>8</sup>D-Toluene, selected as the IS, sampling tools, whose content was analyzed by means of GC–MS for NTDs while TD-GC-MS was used for the analysis of STA and TFMEs. The prototype showed a low background emission, a limited carry-over (<2%) and an acceptable inter-operator and inter-day variability (<15%). Such performances are considered adequate in view of the complexity of biological specimens, such as breath [25]. Notably, the prototype permits to prepare several IS stock gaseous solutions to improve quantitation of the analytes [26, 27]. Finally, modularity is a further prototype strength point. The custom-made board based on ATmega328-pu allows the easy integration of additional digital sensors, e.g. a sensor to monitor CO<sub>2</sub> breath levels and sample selected breath fractions without contamination from ambient air during inhalation [28–30].

## Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

## Acknowledgments

S R A is gratefully acknowledged for making SIFT-MS available. Tobias Bruderer and Matyas Rypszám are acknowledged for their helpful suggestions in designing the tests.

## ORCID iDs

F M Vivaldi  <https://orcid.org/0000-0003-1304-9893>

T Lomonaco  <https://orcid.org/0000-0002-1822-7399>

F Di Francesco  <https://orcid.org/0000-0002-9285-1595>

## References

- [1] Harshman S W *et al* 2016 Storage stability of exhaled breath on Tenax TA *J. Breath Res.* **10** 046008
- [2] López P, Batlle R, Nerin C, Cacho J and Ferreira V 2007 Use of new generation poly(styrene-divinylbenzene) resins for gas-phase trapping-thermal desorption *J. Chromatogr. A* **1139** 36–44
- [3] Ras-Mallorquí M R, Marcé-Recasens R M and Borrull-Ballarín F 2007 Determination of volatile organic compounds in urban and industrial air from Tarragona by thermal desorption and gas chromatography–mass spectrometry *Talanta* **72** 941–50
- [4] Augusto F, Koziel J and Pawliszyn J 2001 Design and validation of portable SPME devices for rapid field air sampling and diffusion-based Calibration *Anal. Chem.* **73** 481–6
- [5] Eom I-Y, Tugulea A-M and Pawliszyn J 2008 Development and application of needle trap devices *J. Chromatogr. A* **1196–1197** 3–9
- [6] Qin T 1997 A simple method for the trace determination of methanol, ethanol, acetone and pentane in human breath and in the ambient air by preconcentration on solid sorbents followed by gas chromatography *Talanta* **44** 1683–90
- [7] Woolfenden E 2010 Sorbent-based sampling methods for volatile and semi-volatile organic compounds in air *J. Chromatogr. A* **1217** 2674–84
- [8] Detlev H and Lee V 1995 Water adsorption capacity of the solid adsorbents Tenax TA, Tenax GR, Carbotrap, Carbotrap C, Carbosieve SIII, and Carboxen 569 and water management techniques for the atmospheric sampling of volatile organic trace gases *Anal. Chem.* **67** 4380–6
- [9] Miekisch W, Trefz P, Bergmann A and Schubert J K 2014 Microextraction techniques in breath biomarker analysis *Bioanalysis* **6** 1275–91
- [10] Mills G A and Walker V 2000 Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials *J. Chromatogr. A* **902** 267–87
- [11] Lord H L, Zhan W and Pawliszyn J 2010 Fundamentals and applications of needle trap devices *Anal. Chim. Acta* **677** 3–18
- [12] Biagini D *et al* 2017 Determination of volatile organic compounds in exhaled breath of heart failure patients by needle trap micro-extraction coupled with gas

- chromatography-tandem mass spectrometry *J. Breath Res.* **11** 047110
- [13] Lomonaco T et al 2020 Release of harmful volatile organic compounds (VOCs) from photo-degraded plastic debris: a neglected source of environmental pollution *J. Hazard. Mater.* **394** 122596
- [14] Lou D-W, Lee X and Pawliszyn J 2008 Extraction of formic and acetic acids from aqueous solution by dynamic headspace-needle trap extraction *J. Chromatogr. A* **1201** 228–34
- [15] Bruheim I, Liu X and Pawliszyn J 2003 Thin-film microextraction *Anal. Chem.* **75** 1002–10
- [16] Cordero C, Sgorbini B, Liberto E, Bicchi C and Rubiolo P 2009 Stir bar sorptive extraction (SBSE) and headspace sorptive extraction (HSSE): an overview *LCGC North Am.* **27** 376–90
- [17] Marín-San Román S, Carot J M, Sáenz de Urturi I, Rubio-Bretón P, Pérez-Álvarez E P and Garde-Cerdán T 2022 Optimization of thin film-microextraction (TF-SPME) method in order to determine musts volatile compounds *Anal. Chim. Acta* **1226** 340254
- [18] Soini H A, Bruce K E, Klouckova I, Brereton R G, Penn D J and Novotny M V 2006 *In situ* surface sampling of biological objects and preconcentration of their volatiles for chromatographic analysis *Anal. Chem.* **78** 7161–8
- [19] Biagini D, Lomonaco T, Ghimenti S, Onor M, Bellagambi F G, Salvo P, Di Francesco F and Fuoco R 2019 Using labelled internal standards to improve needle trap micro-extraction technique prior to gas chromatography/mass spectrometry *Talanta* **200** 145–55
- [20] Sanchez J M 2014 Effects of packing density, flow and humidity on the performance of needle trap devices *J. Chromatogr. A* **1369** 18–25
- [21] Mansour E, Vishinkin R, Rihet S, Saliba W, Fish F, Sarfati P and Haick H 2020 Measurement of temperature and relative humidity in exhaled breath *Sens. Actuators B* **304** 127371
- [22] Španěl P and Smith D 1998 Selected ion flow tube studies of the reactions of H<sub>3</sub>O<sup>+</sup>, NO<sup>+</sup>, and O<sub>2</sub><sup>+</sup> with several aromatic and aliphatic hydrocarbons *Int. J. Mass Spectrom.* **181** 1–10
- [23] Koutras M V, Bersimis S and Maravelakis P E 2007 Statistical process control using Shewhart control charts with supplementary runs rules *Methodol. Comput. Appl. Probab.* **9** 207–24
- [24] Nelson L S 1984 The Shewhart control chart—tests for special causes *J. Qual. Technol.* **16** 237–9
- [25] Thekedar B, Szymczak W, Höllriegl V, Hoeschen C and Oeh U 2009 Investigations on the variability of breath gas sampling using PTR-MS *J. Breath Res.* **3** 027007
- [26] Sysi-Aho M, Katajamaa M, Yetukuri L and Orešič M 2007 Normalization method for metabolomics data using optimal selection of multiple internal standards *BMC Bioinform.* **8** 93
- [27] Bijlsma S, Bobeldijk I, Verheij E R, Ramaker R, Kochhar S, Macdonald I A, van Ommen B and Smilde A K 2006 Large-scale human metabolomics studies: a strategy for data (pre-) processing and validation *Anal. Chem.* **78** 567–74
- [28] Doran S L F, Romano A and Hanna G B 2017 Optimisation of sampling parameters for standardised exhaled breath sampling *J. Breath Res.* **12** 016007
- [29] Maurer F, Wolf A, Fink T, Rittershofer B, Heim N, Volk T, Baumbach J I and Kreuer S 2014 Wash-out of ambient air contaminations for breath measurements *J. Breath Res.* **8** 027107
- [30] Salvo P, Ferrari C, Persia R, Ghimenti S, Lomonaco T, Bellagambi F and Di Francesco F 2015 A dual mode breath sampler for the collection of the end-tidal and dead space fractions *Med. Eng. Phys.* **37** 539–44