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Analytical challenges in breath analysis and its application to exposure monitoring

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There is an increasing interest in the use of breath analysis for monitoring human physiology and exposure to toxic substances or environmental pollutants.

This review focuses on the current status of the sampling procedures, collection devices and sample-enrichment methodologies used for exhaled breath-vapor analysis. We discuss the different parameters affecting each of the above steps, taking into account the requirements for breath analysis in exposure assessments and the need to analyze target compounds at sub-ppbv levels.

Finally, we summarize the practical applications of exposure analysis in the past two decades.

Keywords: Breath analysis; Environmental pollutant; Exposure; Human physiology; Monitoring; Sample collection; Sample enrichment; Solid-phase microextraction (SPME); Toxic substance; Volatile organic compound (VOC)

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1. Introduction

Although in ancient Greece physicians already knew that the specific odor of exhaled breath could be associated with certain diseases, it is only today that breath analysis is beginning to find a wide range of practical applications. Interest in the analysis of volatile organic compounds (VOCs) in breath has increased significantly since the early 1970s, when Pauling et al. [1] reported a gas-chromatography (GC) method for the analysis of breath samples.

Breath analysis has the great advantage of being a non-invasive technique when monitoring the physiology of a person or exposure to toxic substances or environmental pollutants. Compared to blood or urine analysis, breath analysis is more acceptable to potential study participants and repeated sampling is less likely to be perceived as unpleasant. The measurement of gas-phase analytes is also simpler in a gas matrix (e.g., breath) than in more complex biological matrices.

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Breath samples can be used in two important fields:

[1] clinical diagnosis to analyze volatile compounds generated in the organism and eliminated through exhaled breath (endogenous compounds); and,

[2] exposure analysis in order to have fast, accurate information regarding the levels of potentially noxious inhaled VOCs reaching the blood stream (exogenous compounds).

Clinical diagnosis, which has received the greatest interest due to its potential to detect disease states in a simple and non-invasive manner, has already been extensively reviewed [2–7] and is beyond the scope of this review.

Exposure assessment is of great interest in the determination of toxic substances in indoor environments as people in western populations spend more than 70–80% of their time indoors [[8]] and can be exposed to a range of indoor pollutants that may have adverse effects on health. Exhaled breath has been analyzed to determine personal exposure to solvents and other volatile organic compounds (VOCs) [5,9–29]. Most information on VOC toxicity is based on exposure in industrial environments that typically have high levels of pollutants (ranging from hundreds of $\mu g/m^3$ to mg/m^3) or has been established from animal and controlled studies with high concentrations. Levels in most indoor environments are below the exposure limits required to demonstrate measurable health impacts [30,31]. Although there is as yet no solid evidence of a health risk at the low levels normally detected in homes (from a few $\mu g/m^3$ to ng/m^3), some VOCs are well-established carcinogens and others, due to poorly understood mechanisms, may be allergenics [32]. Unfortunately, the few epidemiological studies conducted in these conditions have given us insufficient data to elucidate any possible relationship between exposure to VOCs in non-industrial environments and their effect on human health [33], even in the case of repeated, prolonged contact [17].

One of the main problems associated with the analysis of exposure in non-industrial environments is the low concentration of contaminants. Taking into account the levels usually detected in these environments, analytical methodologies with limits of detection (LODs) <1 $\mu g/m^3$ are required [18,30,31]. The limited volume of breath samples requires the use of highly sensitive methods to achieve appropriate limits for the detection of target compounds.

It is necessary to distinguish between two different types of samples when we refer to exhaled breath:

(1) exhaled breath vapor (EBV); and,

(2) exhaled breath condensate (EBC).

Only volatile compounds form EBV. The main fraction (>99%) comprises a mixture of nitrogen, oxygen, CO_2, water vapor, and inert gases. The remaining fraction (<100 ppmv) is formed by a mixture of hundreds of VOCs in a wide range of concentrations (ranging from a few ppmv to pptv) [5,6].

However, EBC is more complex, as it is a mixture of the breath expired from the lungs and the aerosolized droplets emerging with the breath. EBC contains both volatiles and non-volatiles, and these must be recognized as separate entities with different properties [34]. But, it has been found that conventional EBC-collection methods (e.g., Rtube at -80ºC) yield significantly lower sensitivity in the analysis of VOCs than specific EBV-concentration methodologies {e.g., solid-phase microextraction (SPME) [35]}. When VOCs are the analytes of interest, EBV sampling is preferred.

The qualitative composition of EBV varies considerably from person to person and depends on the metabolism of each individual (endogenous compounds) and on the

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environment around each person (exogenous compounds). The main VOCs present in a healthy person's breath are acetone, isoprene, methanol, and ethanol, which are produced endogenously in core metabolic processes [36]. All other VOCs are present at very low levels, from a few parts per billion (ppbv) to below parts per trillion (sub-pptv) (i.e. $\mu g/m^3$ to ng/m^3).

2. Breath-sampling procedures

One of the main problems when dealing with breath analysis is the limited volume of sample that can be obtained. Moreover, breath needs to be collected under careful conditions that include monitoring the breathing [37]. The average total lung capacity of an adult human male is about 6 L of air, but only a small amount of this capacity is used during normal breathing. In each expiration, almost 500 mL of breath is expired [5,13]. The first portion is "dead space air", which comes from the mouth, trachea and bronchi, so it does not involve a gaseous exchange between air and blood. The remaining fraction is "alveolar air", which comes from the lungs, so it does include a gaseous exchange between air and blood. Exhaled breath is a mixture of dead space and alveolar air.

Preliminary studies sponsored by the US Environmental Protection Agency (EPA) in the 1980s relied upon a spirometer and the collection of a 40-L volume of breath [38]. This method allowed the detection of low levels of VOCs but was cumbersome and had many drawbacks. More recent EPA studies breath samples of only 1 L.

The volume of breath sample that is usually collected currently ranges from a few mL when VOCs are directly retained into a sorbent device [23,39–42] to 1 L when breath is collected in a gas-sampling container [12,13,16,20–22,25,27,29,43–46].

To collect more than 0.5 L of sample, it is necessary to use forced-expiratory sampling or to collect samples from tidal breathing over several expirations. Forced-expiratory sampling, a common sampling methodology used in different studies [44,45], is a simple procedure that does not require complex instrumentation. This methodology has been proposed to obtain a steady flow of representative alveolar air, but it has many drawbacks:

(1) it is highly dependent on the volunteer's cooperation and effort;
(2) breath-holding with the lung full or partially emptied gives different results; and,
(3) there is no control of the volunteer's breathing.

Despite its widespread use in non-clinical studies, this should not be recommended as a sampling procedure for quantitative analysis.

Sampling by collecting different exhalations during tidal breathing would seem to be the most reliable methodology, despite breathing patterns being irregular and the existence of random fluctuations in breathing frequency and intensity [47], making it necessary to collect breath samples from a series of cycles. The collection of breath during tidal breathing is not without difficulty, as the hyperventilation that is typical when a person is asked to breathe normally [37] changes the distribution of molecules across the alveolar-capillary junction over time. It has been demonstrated that the concentration of compounds varies considerably depending on the type of ventilation at the moment of sampling (hypoventilation, hyperventilation and normal ventilation), leading to results that are difficult to interpret [48]. Samples should be obtained during conditions of normal ventilation, which requires introducing the volunteer to the procedure and encouraging the adoption of a relaxed natural and regular breathing profile.

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In tidal breathing, different types of samples can be collected depending on the aim of the study [2,49]:

(1) mixed expiratory or total breath sampling;
(2) time-controlled sampling (i.e. sampling over a predetermined time after the beginning of the expiration); and,
(3) alveolar or end-tidal sampling.

In mixed expiratory sampling, there is no need to identify each fraction and the whole expired air is directly collected into an appropriate device. In clinical work, this sampling procedure should not be used, as it is subject to dilution and contamination by exogenous substances from the "dead space air". However, the analysis of this fraction is required when substance concentrations in the airways are of interest [49], as is the case in exposure analysis.

Time-controlled sampling presents less dilution and contamination from airways than mixed expiratory sampling, but large variations are found when repeated samples from the same individual are evaluated [49]. For this reason, this sampling methodology should also be avoided in clinical practice.

Alveolar sampling is more complex instrumentally and the difficulties in consistently capturing alveolar air samples have long been recognized [50]. This technique has the advantage for clinical purposes that there is no contamination from the dead-space volume. Moreover, the concentration of endogenous substances is 1–3 times greater than in mixed expiratory samples [17,49]. As a result, it is easy to identify and to quantify blood-borne substances. This is the sampling procedure most recommended for clinical applications.

The large amount of variables indicated and the fact that many different sampling methodologies are used for exhaled air makes it difficult to compare results [17]. It is therefore desirable to find a standardized system to allow comparison [37]. Moreover, a standardized, reproducible breath sample is required for quantitative analysis to avoid the proportion of alveolar to tidal air varying from sample to sample [50]. The only way to obtain reliable, comparable results is to normalize samples at alveolar concentration levels [2,37,49]. Different methods have been evaluated to determine when the alveolar plateau is reached during a single expiration (e.g., monitoring CO_2, O_2 or breath temperature) [50]. The best results are obtained by the simultaneous determination of CO_2 in expired breath as a corrective factor [37]. A CO_2 controller is commonly used as CO_2 concentrations are higher and practically constant in the alveolar phase [49–51]. When CO_2 levels increase and plateau out, portions of breath can be obtained by using a syringe (single breath sample) [49] or connecting a collection device to the system (several breaths) [49,51].

3. Breath-collection devices

Since the end of the twentieth century, different methods for the direct reading (real-time analysis) of breath samples {e.g., laser spectrometry, selected ion flow-tube (SIFT), atmospheric pressure ionization (API), proton-transfer reaction (PTR), ion-mobility spectrometry (IMS), and sensors} have appeared with promising results [4,15,16,52–60]. Some of these real-time methodologies allow responses to be obtained in less than 1 s [52]. Although most studies indicate LODs as being at the $\mu g/m^3$ level [15,16,52,54–58], some have proposed LODs at the ng/m^3 level [52,59,60].

Reynolds et al. [55] have proposed the coupling of a thermal desorption unit to a real-time IMS instrument, as many compounds of interest are present at concentrations

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below the LODs for direct breath analysis. Unfortunately, many of these methodologies require complex, non-portable and expensive instrumentation, which limits their applicability for on-site determinations in exposure analysis.

Indirect methods involving collection devices to obtain and to transport the samples are less expensive, and, at the moment, seem to be the most appropriate methodology for obtaining on-site breath samples. The sampling, transport and storage of exhaled breath are therefore critical steps in the whole analytical process. The preservation of the original sample composition is a challenge for gas compounds, as losses (e.g., by diffusion), adsorption (e.g., in the surface of the containers) and reactions can occur, leading to the formation of artifacts. Thus, the selection of the most appropriate container is essential in EBV sampling. Samples can be collected using different devices (e.g., canisters, sampling bags and sorbent materials) [17,61].

Canisters are used for collecting breath samples [11–13,16,21,22,24,43,62], but they have the disadvantage of being expensive, needing to be evacuated before sampling and requiring sophisticated equipment for cleaning [63,64]. Some authors have suggested that passivated stainless-steel canisters are extremely durable for breath storage, and many VOCs remain stable within these canisters for periods of 30 days or longer without any significant degradation [43]. However, Batterman et al. [65] evaluated the stability of some aldehydes and terpenes in electropolished canisters and found that recoveries for all terpenes and most aldehydes evaluated dropped substantially within the first hour, followed by a more gradual decrease later.

Glass bulbs can also be used for breath sampling [15,23,28,46,66]. However, they are fragile, require silanization to deactivate the interior glass surface [46,67], and must be evacuated before sampling. Moreover, losses of volatile compounds have also been observed when glass bulbs are used as containers, although at lower rates than with polymer bags [46]. In some cases, polymeric chambers [39,40,68,69] have been used to collect breath samples. Unfortunately, no information about losses and stability has been recorded. Some losses of volatile compounds are to be expected due to the polymeric structure of the chamber walls.

The most common methodology for breath collection is to use polymer sampling bags, due to the ease with which they can be manipulated, their reduced cost and the possibility of reuse. These bags must be made of inert materials to avoid both diffusion and reactions between the compounds and the bag. The most commonly used material is Tedlar [10,20,25–27,29,44,61,70–74] but other materials such as Teflon [9,53,74,75], FlexFoil [74], and Nalophan [74] are also used.

Prior to being used for the first time or being reused, bags must be thoroughly cleaned by flushing with pure inert gas to remove adsorbed compounds. This step plays a crucial role in the storage of gas samples [73,74]. Unfortunately, all commercial polymers suffer from diffusion and adsorption of volatile compounds [29,70,71,73,74,76], and temperature and storage time have a significant effect on the integrity of the gas samples [72]. Although Tedlar bags are the most common choice for breath analysis, they also present the most significant background contamination. When Tedlar, Teflon, FlexFoil and Nalophan polymers were compared [74], only Tedlar polymer emitted contaminants in blank tests. The main contaminants detected in Tedlar bags are N,N-dimethylacetamide and phenol, which are both solvents that are used in the production of the film [72,73,76]. Other contaminants that have been detected in these bags are carbonyl sulfide and carbon disulfide [74]. It is usually recommended that breath samples should be analyzed as soon as possible after sampling.

A common commercial device for breath sampling is Bio-VOC [19,77–79]. This device is based on the collection of the last 100–150 mL of an expired sample.

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Immediately after taking a sample, a valve is opened and the collected breath is transported through an appropriate sorbent material in order to retain the VOCs. The gas sample only remains in the container for a few seconds with this device and no losses are expected. Although the manufacturers state that only alveolar air is collected, there is no control of the subject's breathing and CO_2 levels.

Other similar devices that have been used to collect breath samples are modifications of the Haldane-Priestley tube [80]. One option comprises an insulated aluminum tube that holds a collapsible Tedlar sampling bag (85 mL). After collecting end-tidal breath in the bag, an adsorption tube is attached to the sampling tube to adsorb the volatiles in breath [81–83]. A recent modification comprises an insulated aluminum tube maintained at 40–45ºC to prevent condensation of the water vapor [84]. The sample is then transferred to a Tenax TA adsorption trap using a 50-mL gas syringe.

Different direct sampling methodologies were recently developed to integrate sampling and pre-concentration into one single step, which can avoid the problems related to storage in containers. These methodologies are based on the direct collection of target VOCs on a sorbent material, which presents better stability and permits longer storage times. A modified holder connected to a SPME fiber has been developed [67,69]. The use of hydrophobic membranes to eliminate water vapor and impurities followed by pre-concentration in a sorbent trap has also been proposed [39,40]. The use of a device called SnifProbe, which is based on a small length of capillary or porous-layer open tubular column for sample collection, has been proposed [85]. An adaptive breath sampler to collect breath directly in a sorbent tube is another option [47]. Most recently, needle-trap devices (NTDs) were described [41,42,49,86].

4. Sample enrichment

The low concentrations of VOCs in breath samples make it necessary to employ a pre-concentration technique before analysis with the GC with mass spectrometry (GC-MS) or GC with flame-ionization detection (GC-FID) analytical techniques used for conventional breath analysis. There are two main methodologies for this purpose: SPME and concentration on solid sorbents.

4.1. Solid-phase microextraction (SPME)

Different procedures are followed in SPME (Table 1) [3,17,35,46,61,66,67,69–71,87,88]. Sometimes the fiber is inserted into the bag or glass bulb containing the total volume of breath collected for a predetermined period of time [46,66,70,71], or a fixed and small volume of the sample is transferred inside a sealed vacuum headspace vial before inserting the SPME fiber into the vial [87,88].

The small volume of the stationary phase is an advantage of SPME when sample sizes are not large [89]. The sensitivity of SPME is not as dependent on the volume of the sample as conventional concentration on solid sorbents. LODs are commonly in the low-ppbv range when SPME is applied to breath samples (Table 1). Unfortunately, this limits the applicability of SPME when target compounds have to be detected at lower levels. For example, 2,5-dimethylfuran, a promising breath biomarker for determining smoking status or continuous exposure to environmental tobacco smoke, has to be detected at the low-pptv range in breath samples if it is to be found some hours after contact with tobacco smoke [20,29,86]. Analyses performed in our laboratory showed that SPME failed to detect this compound just a few minutes after smoking a cigarette,

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whereas the compound was detected for more than 24 h after smoking using a multi-bed sorbent capillary trap.

It has been found that the water content of a sample has a significant effect on the SPME sorption process when direct breath analysis is performed [67]. For those coatings where absorption is the dominant process (e.g., PDMS), extraction efficiency is not affected by the water content. However, there is a significant change in the extraction efficiency in the case of adsorption mechanism-based coatings [67,90–92] due to the competition with water molecules and other VOCs for the active sites of the sorbent material. Calibration standards should be prepared at the same relative humidity (RH) as samples, and sampling times should be chosen carefully to avoid competitive adsorption that can lead to inaccurate quantification [67,70]. Levels of acetone, the main VOC detected in healthy people, vary significantly from one person to another, usually 200–800 ppbv (but up to 3500 ppbv in diabetics) [93,94]. This wide variation, together with the fact that other VOCs are usually present at significantly lower levels (2-3 orders of magnitude less than acetone) [20], may result in losses by competitive adsorption if accurate sampling conditions are not chosen.

4.2. Concentration on solid sorbents

Pre-concentration on solid sorbents followed by thermal desorption (TD) is the most frequent method for the analysis of VOCs in breath samples [9–13,16,19-28,43–45,47,55,62,75,81–85,95,96]. Sorbent traps present the advantages that they can be prepared on a micro-scale and coupled on-line with a GC system to allow near real-time measurements [97–101], and the sorbent configuration can be easily changed to adapt to different compounds.

LODs obtained by sorbent-trap techniques strongly correlate with the volume of sample analyzed. Thus, LODs obtained applying this technique decrease significantly compared to SPME limits when a large volume of a breath sample can be collected. LODs obtained with the EPA-TO-14 method in the analysis of 8-L breath samples were one order of magnitude lower than those obtained with SPME [70]. The use of a micro-trap allows LODs in the low-pptv range to be reached with samples volumes up to 1 L [20,27].

The most common, simple sorption trap is based on a single adsorbent. In the case of exposure analysis, the most common sorbent used is Tenax [9,10,19,23,28,75].

However, the wide range of VOCs present in breath results in no single sorbent being capable of adsorbing all the compounds present in breath samples, so a multi-component sorbent is necessary to complete screening and determination [25,44,64,95–97,100,101].

The sorption and the desorption of VOCs in carbon-based sorbents is important, as they determine the injection-plug width and the ability to perform quantitative studies. An important source of error when sorbent traps are used is the formation of artifacts caused by degradation reactions of both adsorbed analytes and the adsorbent itself during storage of adsorbent tubes [102,103]. These errors, which take on particular importance when very low concentrations of the target compounds are expected, can be reduced by using on-line traps [102,103].

A further difficulty in the use of sorbent traps is that the high temperatures needed for quantitative desorption can lead to the thermal decomposition of some of the trapped compounds [104,105] (Fig. 1). The problem of degradation is more important with conventional desorption equipment, as a second pre-concentration stage is required to refocus the solutes in the analytical column. This is frequently done by cryogenic trapping, which can also result in analyte loss and the formation of artifacts [106].

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Furthermore, the sorbent material itself can generate artifacts by degradation at high temperatures [64].

In order to simplify the desorption process and to overcome the problems of decomposition, different micro-traps have been developed. These micro-traps eliminate the need for a second cryofocusing stage and allow near real-time measurements [25,27,44,45,97–101]. The configuration of the micro-traps allows much greater concentration factors than those obtained with conventional thermal desorption instruments and requires a smaller amount of sample for LODs in the pptv range to be reached [25,27,44,45].

NTDs represent a further improvement in capillary traps for breath analysis [41,42,86]. These devices allow direct thermal desorption inside a GC injector (equivalent to SPME) and have performed as well or better than SPME when both methods have been compared [108–111]. The small i.d. of the NTDs results in high linear flows through the sorbents that limit the maximum flow rate that can be used during sampling collection (≤15 mL/min) [86]. Given this, NTDs allow for limited volumes of sample to be collected in a reasonable period of time [41,49].

For the sorption process, it is important to take into account the water intake of the sorbents, as this can affect the quantitative analysis of VOCs. Graphitized carbon blacks and porous organic polymer adsorbents allow a high percentage of water vapor in the sample to pass through the traps during sampling without significant loss of the target compounds [111,112]. Unfortunately, if highly volatile compounds are on the target list, strong adsorbents (carbon molecular sieves) are required in order to retain them and large amounts of water are retained at the same time. The simultaneous trapping of water vapor can cause various problems:

(1) accumulation as ice during cryogenic pre-concentration;
(2) reduction in the adsorption efficiency during sampling on solid adsorbents;
(3) the possible loss and transformation of organic trace gases in the water/ice matrix;
(4) the freezing of water in the trap or GC column during cryogenic oven cooling may result in blockages that interrupt the carrier gas flow; and,
(5) a large water background may shift retention times and pose problems during detection, especially in the case of MS detectors [111].

Different options have been proposed to limit the water-vapor problem [111].

One option is to pass the sample flow through a trap containing a drying agent or a membrane (e.g., Nafion). The membrane allows water to permeate through it, but it also permits the passage of other light, polar, volatile compounds, resulting in losses of highly volatile compounds [64].

Another simple option is to dry the sample with a dry inert gas after the sample concentration is completed [63,111]. In this case, there are also limitations due to the possible loss of VOCs or the introduction of contaminants [64,111,112].

A further option is to heat the adsorbent during sampling [64,111], but this results in most volatile compounds not being quantitatively retained by the sorbent [44] (Fig. 2).

The simplest way to reduce the water problem involves reducing the volume of sample so as to reduce the amount of water vapor in the sample to below the thresholds for the proper use of the analytical instrumentation [63,111]. However, this option is only possible where small amounts of breath samples are collected.

Breakthrough volume (BV) is another important issue to take into account when sorbent traps are used. In the case of commercial thermal desorption devices, the amount of sorbent inside the trap is typically a few hundred mg. Studies into BVs in the case of commercial traps have found that they range from hundreds to thousands of L/g at $\mu g/m^3$ levels [113]. This suggests that it is difficult to achieve the BV in breath

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analysis if appropriate sorbents are selected, since the volume of sample usually evaluated is <1 L.

The BV becomes a significant issue when micro-traps are used [44,97,99–101]. The amount of sorbent that it is possible to fit in the micro-traps is in the tens of mg range, and BVs <1 L have been obtained at the 100-ppb level [101]. The appropriate selection of the sorbent material is even more important, as BVs can vary from <0.5 L to >10 L, depending on the sorbent material [100,101]. When working with samples (e.g., breath) that contain hundreds of different compounds, the use of a multi-bed trap enables appropriate BVs for each of the compounds studied [44,97,101].

In the case of NTDs, the small i.d. diameter of the needles (<1 mm) results in amounts of sorbent of <2 mg. Given this, the BV, which is typically <150 mL, is even more important with these devices [114–118]. NTDs seem only to be the most appropriate choice when sample volumes are relatively low (e.g. <100 mL).

5. Applications in exposure analysis

Five categories of study can be found in the literature:
(1) simulations in controlled chambers [21,77,82,83];
(2) swimming and domestic water activities [12,24,68,78,84,119];
(3) services related to mechanical vehicles [22,23,28,69,120,121];
(4) solvents and volatile compounds in the workplace [15,19,79,122–124]; and,
(5) active and passive smoking [16,20,25,27,29,51,86,125].

Most of the studies are focused on finding reliable exposure biomarkers.

It is difficult to compare the different results obtained in these studies and to assess the influence that the preparation methodology or analytical techniques may have had on them as they employ widely varying parameters. Most of the studies are based on the collection of forced-expiratory samples. Although the authors often refer to this as alveolar sampling, the continuous CO_2 monitoring that is required to assure that the breath sample is collected during the alveolar phase was applied in only one study [51].

As an alternative, Pleil et al. [22] measured the CO_2 concentration in samples collected in canisters. These authors reported that, if a breath sample is collected from the alveolar phase, the CO_2 concentration reaches a relatively constant value (3.0–5.5%). Lower values were associated with the dilution of the alveolar breath with dead-space air or ambient air.

Furthermore, there is considerable variation in the breath-collection devices used (e.g., canisters, glass bulbs, Tedlar bags, and sorbent tubes) and in the sample volumes tested. In only two studies were real-time devices used for the direct detection of VOCs in breath [15,16]. In practically all the studies, preconcentration and analysis of target VOCs were performed with a TD-GC-MS system, which obtained LODs in the 0.1–10 $\mu g/m^3$ range. Only in those studies where micro-traps [20,25,27,29,45,86] or real-time instruments [52,59,60] were used did LODs decrease to the ng/m^3 level.

5.1. Simulations in controlled chambers

These studies aim to simulate conventional exposure situations in order to investigate whether breath measurements can be used as a surrogate for blood measurements. The main drawback of simulation studies is that controlled chambers are designed to assess exposure at levels that are equivalent to the threshold limit values and so results cannot be extrapolated to non-exposed people.

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Exposure to trichloroethene levels was evaluated from controlled inhalations at 100 ppmv for 4 h [21]. Sampling was performed with 1-L canisters and analyses were based on the EPA TO-14 method using a thermal desorption unit with two cryogenic traps for preconcentrating and focusing, followed by GC-MS. A model was used to predict blood levels from breath-elimination curves and blood/breath-partition coefficients. The results obtained gave a mean ratio of blood level calculated:measured of 0.98. The study concluded that about 78% of thrichloroethene entering the subjects was metabolized, stored or excreted through routes other than exhalation.

Exposure to trimethylbenzene was performed in a controlled volunteer study where participants were exposed for 4 h to this compound at 25 ppm in a laboratory controlled-atmosphere facility [77]. A commercial Bio-VOC breath-sampling device using Tenax as the sorbent was used and analyses were performed with a thermal desorption (TD)-GC-MS system. Trimethylbenzene, which is largely produced by inhalation, was found to be rapidly adsorbed into the blood stream. The analysis of exhaled breath and a metabolite in urine revealed that the compound was incompletely eliminated in these two ways.

Breath analysis and physiologically-based pharmacokinetic (PBPK) modeling were used to estimate exposure to *m*-xylene at 50 ppmv [82] and 40 ppmv [83] in laboratory controlled-atmosphere facilities for 4-h and 12-h periods. In both cases, a modified Haldane-Priestly tube coupled to an adsorption trap was used to collect end-tidal samples, and analyses were performed with a TD-GC-MS system. Loizou et al. [82] analyzed combined inhalation and dermal exposure and dermal-only exposure. They derived a one-skin-compartment PBPK model, which was able to predict post-exposure decays of *m*-xylene in breath. Exhaled concentrations of *m*-xylene were ~10 times lower than atmospheric concentrations and a post-exposure elimination half-life of 30 min was found independently of the exposure time evaluated, suggesting that steady-state conditions are reached in the blood during constant exposure at 50 ppmv for under 4 h.

When dermal-only exposure was evaluated, exhaled breath levels were >60 times lower than those obtained with dermal and inhalation exposure. In this case, a skin:air partition coefficient of 30 was found to fit with the proposed model. The authors concluded that for *m*-xylene vapor the dermal route contributes 1.8% of the total dose.

McNally et al. [83] also used PBPK modeling to assess *m*-xylene exposure for 4 h at 40 ppmv. They found that the PBPK model developed was unable to fit breath decay independently but concluded that the integration of PBPK modeling, global sensitive analysis, Bayesian inference, and Makov chain Monte Carlo simulations resulted in a powerful approach for exposure reconstruction from biological monitoring data.

5.2. Swimming and domestic water activities

Trihalomethanes (THMs) are important contaminants in indoor and outdoor swimming pools and in domestic water activities. They are formed as a result of the combination of residual organic matter and chlorine-based disinfection products used in water-supply systems. Lindstrom et al. [12] collected forced expiratory breath samples with 1-L canisters before, during and after a 2-h training period. Analyses were based on the EPA TO-14 method with cryogenic traps. They suggested that the dermal route of exposure was even more important than inhalation.

Other studies using a Bio-VOC device for sampling followed by TD-GC-MS analysis [78] or by collecting breath samples in 34-cm^3 glass tubes followed by direct injection in a GC-MS [119] also found that dermal uptake for these compounds was significant. A face-mask with a polyethylene tube connected to a Tenax trap was used in a study specifically designed to confirm the dermal uptake of chloroform and two

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haloketones during bathing [68]. It was found that haloketones are less permeable through skin than chloroform.

Canister sampling followed by analysis based on the EPA TO-14 method was used in a study to assess the breath levels of THMs during domestic use [24]. A significant increase in the target THMs was observed during bathing and showering but no significant increases were found in breath levels as a result of other domestic water use activities (e.g., washing clothes or dishes), even though these activities led to a significant increase in the indoor air levels.

Another study that used a modified Haldane-Priestly tube coupled to a Tenax TA sorbent trap and analyzed the compounds with a TD-GC-ECD system [84] also confirmed THM exposure due to showering and swimming activities and found that chloroform was the dominant THM of those that were evaluated (i.e. chloroform, bromodichloromethane, dibromochloromethane, and bromoform).

5.3. Services related to mechanical vehicles

Benzene, toluene, ethylbenzene, and xylene isomers (BTEXs) are common compounds in petrol products. These compounds evaporate easily from the liquid and can be inhaled. Studies using different sampling methodologies {e.g., canisters [22], Tenax sorbent tubes [23], glass bulbs [28], SPME [69], and Tedlar bags [121]} have found that benzene levels in the exhaled breath of people exposed to petrol vapors are always higher than in volunteers who are not exposed. These studies also found large variability in breath-benzene levels for all groups evaluated, but this variability was significantly higher in the case of exposed participants.

In a study using 3-L Tedlar bags to collect two to three complete breaths, which were then analyzed by a TD-GC-MS system, exhaled toluene and xylenes were found to correlate significantly with concentrations found by personal monitoring devices [120]. As a consequence of these results, exhaled breath levels of benzene, toluene and xylenes have been proposed as being suitable for use as biological exposure indices for petrol-station workers. Naphthalene has been found to be eliminated faster from the body than benzene [28].

5.4. Solvents and volatile compounds in the workplace

Occupational exposure to benzene was evaluated in workers of a benzene-production plant during their entire work shift [122]. Forced expiratory breath samples were collected in glass tubes and analyzed with a TD-GC-MS system. Significant differences for benzene levels in breath and blood were obtained between exposed and non-exposed workers. Benzene alveolar retention of around 55% was reported. When workers from different occupations were evaluated using a Bio-VOC device with Tenax TA for sampling, higher concentrations were detected after work shifts [79].

Scheepers et al. [19] analyzed end-exhaled breath (sampling was performed with a BioVOC device and Tenax as the sorbent) and personal exposure to BTEXs of primary-school children from two different zones. They found that industrial activity made a relatively small contribution to exhaled BTEXs. Other factors (e.g., smoking habits, the presence of petrol services and traffic, and the use of consumer products) seem to have a greater influence on exposure to benzene and toluene.

Park and Jo [123] studied the breath of 120 children, comparing those who attended school close to an industrial complex with those who went to school in a low traffic residential area. On analyzing breath samples collected with a canister using a TD-GC-FID system, they found that personal air concentrations in those schools close to the

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industrial complex were higher than in the residential areas and that breath concentrations correlated significantly with personal air levels.

The same group also used the same sampling methodology to study the extent to which the proximity of houses to service stations affected the exposure of housewives to BTEX [124]. Although significant differences were found for outdoor air levels, with higher levels being detected in close proximity to service stations, there were no differences in the indoor air levels. In all indoor areas evaluated, the levels were significantly higher than were found outdoors (indoor-to-outdoor ratios ranged from 1.3 to 4.8). Breath levels only correlated significantly to indoor air levels, but not to outdoor levels. It was concluded that the major parameter for the exposure of housewives was indoor VOC levels. The only significant difference observed in breath levels was attributable to the smoking status, with significantly higher breath levels being found in housewives living in smoking houses for all compounds except *o*-xylene.

Thrall et al. [15] developed a real-time tandem ion trap mass spectrometer for breath analysis and applied the system to measure selected solvents in exhaled air. Benzene and toluene were evaluated in workers from an incinerator, and trimethylbenzene, hexane and methylene chloride were determined from employees in a waste repackaging facility.

5.5. Active and passive smoking

The last category evaluated is of studies devoted to tobacco smoking, exposure to environmental tobacco smoke (ETS) and passive smoking.

Buszewski et al. [51] analyzed 56 VOCs in the alveolar breath of 20 non-smokers, 14 active smokers and 4 passive smokers. They collected samples in 1-L Tedlar bags and applied capnography to confirm that alveolar samples were obtained. Preconcentration was performed by SPME followed by GC-MS analysis.

Van Berkel et al. [125] collected forced expiratory breath samples in 5-L Tedlar bags and analyzed them with a TD-GC-MS system. They identified four VOCs as biomarkers of recent exposure to cigarette smoke: 2,5-dimethylhexane, dodecane, 2,5-dimethylfuran, and 2-methylfuran.

Gordon et al. [16] used a real-time ion trap mass spectrometer to evaluate the breath profiles of benzene, 1,3-butadiene and 2,5-dimethylfuran from smokers and passive smokers after smoking cigarettes in a~~n~~ small unventilated room. The contamination of non-smokers by this exposure was demonstrated by the fact that all three target VOCs were identified in their breath after exposure.

2,5-dimethylfuran was found to be a biomarker of smoking status independently of smoking status [20,25,27,29,45,86]. The use of micro-traps and NTDs has allowed LODs to be lowered to ng/m^3 levels. The evaluation of ETS contamination on public premises confirms this compound to be a robust biomarker of ETS contamination [29]. The compound was also detected in the breath of non-smoking employees working on smoking premises a few hours after the beginning of their work shift.

6. Conclusions

The non-invasive nature of breath analysis and the fact that breath is a less complex matrix than other biological matrices (e.g., blood, urine and tissue) have spurred the use of breath analysis in exposure assessment in recent decades. However, despite the improvements that have been achieved in its application, the technique is still far from being accepted for routine analysis. Further development of the sample-collection

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devices and the sampling mechanisms is required in order to facilitate taking reliable, reproducible samples. Furthermore, portable devices need to be developed to enable the simple, robust analysis of VOCs at sub-pptv levels, so as to be able to determine VOCs accurately in the exhaled breath of non-exposed people. New real-time technologies and methods based on micro-traps and needle traps may well show the way forward in refining our ability to monitor exposure.

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Captions

Figure 1. Chromatograms showing the thermal degradation of α-pinene when the temperature applied to the sorbent trap for desorption is increased. The sorbent trap was heated to 200ºC (a), 250ºC (b), 300ºC (c), 350ºC (d), and 380ºC (e). (u: unknown).

Figure 2. Effect of the temperature applied during the sorption process in the analysis of a breath sample (750 mL exhaled breath). Three-bed trap containing Carboxen 1000, Carbopack X and Carbopack B as sorbent materials. As can be seen, there is a significant decrease in the peak heights for the most volatile compounds (compounds appearing at retention times <200 s) when the trap was heated at 40ºC during the sampling process (b). Sampling at 22ºC (a) yields better sensitivity for the most volatile compounds. Less volatile compounds (r.t. >200 s) are not significantly affected by the change in the trap temperature during the sorption process.

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Table 1. Summary of the principal studies using solid-phase microextraction (SPME) as preconcentration technique and devoted to exposure analysis

Coatings	Target VOCs	LOD	Sampling collection device	Ref.
CAR/PDMS	Isoprene	17 μg/m^3 (SPME) 1 μg/m^3 (sorption)	8 L Tedlar bag SPME inside bag, 10 min at 40ºC	[[70]]
PDMS/DVB	Acetone	0.1 μg/m^3	3 L Tedlar bag (max. storage 6 h) SPME inside bag, 4 min at 40ºC	[[71]]
PDMS	Tetrachoroethylene	300 μg/m^3	125 mL glass bulb (exposed 1 min)	[[66]]
PA PDMS CW/PDMS PDMS/DVB	Ethanol Acetone Isoprene	276 μg/m^3 116 μg/m^3 20 μg/m^3 (PDMS/DVB coating)	Fiber directly to mouth (10 s)	[[67]]
PDMS CW/DVB PDMS/DVB DVB/CAR/PDMS	benzene	6 μg/m^3	Fiber directly to mouth (30 s)	[[69]]
CW/PEG	2-aminoacetophenone	7 μg/m^3	1 L glass bulb (24 h fiber)	[[46]]
CAR/PDMS	Acetone Acetonitrile Benzene n-butane Dimethylsulfide Furan 2-methylfuran Isoprene Limonene Toluene	5 μg/m^3 25 μg/m^3 0.2 μg/m^3 12 μg/m^3 10 μg/m^3 6 μg/m^3 7 μg/m^3 0.6 μg/m^3 11 μg/m^3 0.4 μg/m^3	3 L Tedlar (20 mL vials, 10 min at 37ºC)	[[87]]
CAR/PDMS	43 VOCs	1-90 μg·m^{-3}	3 L Tedlar (20 mL vials, 10 min at 37ºC)	[[87]]

Figure 1

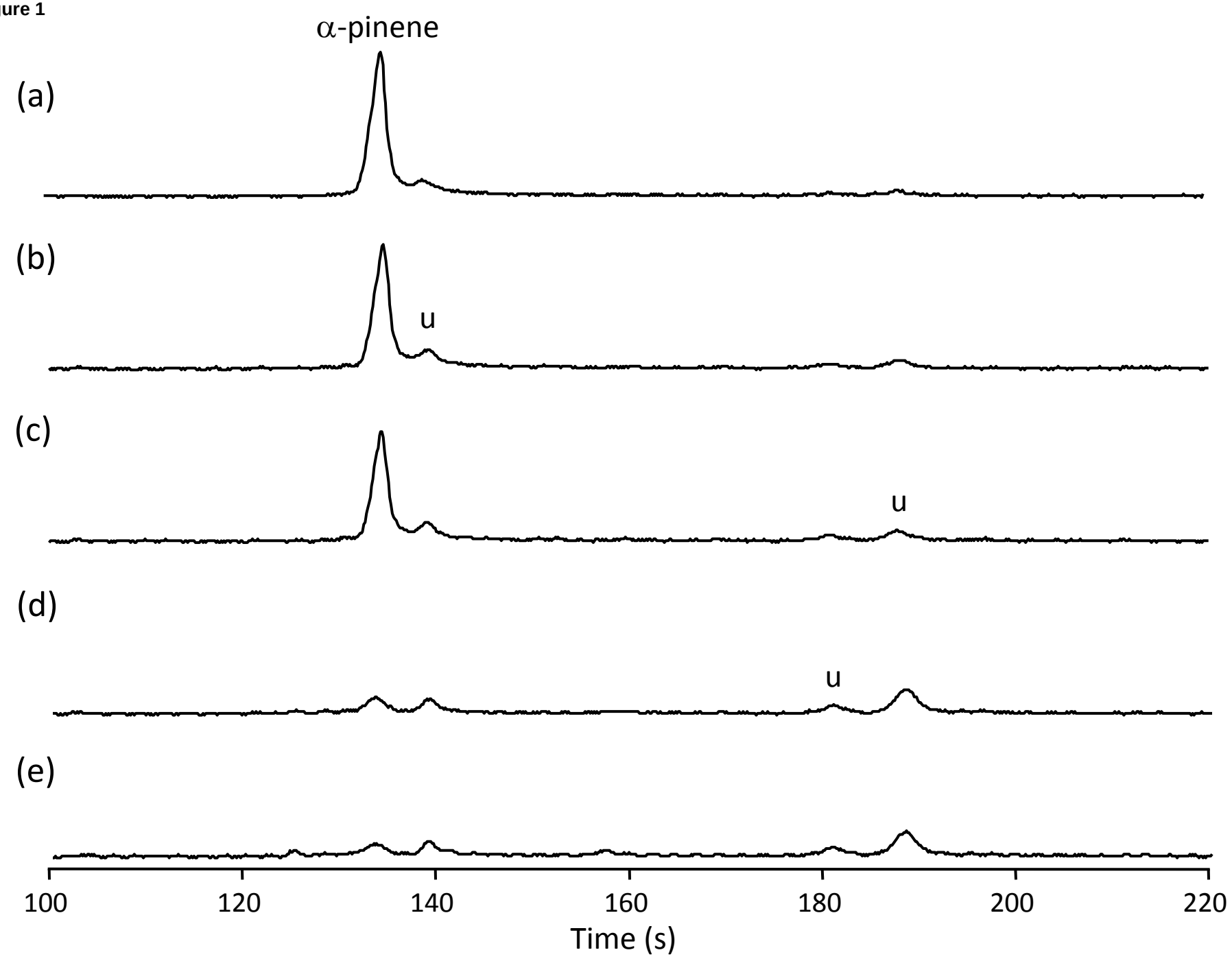
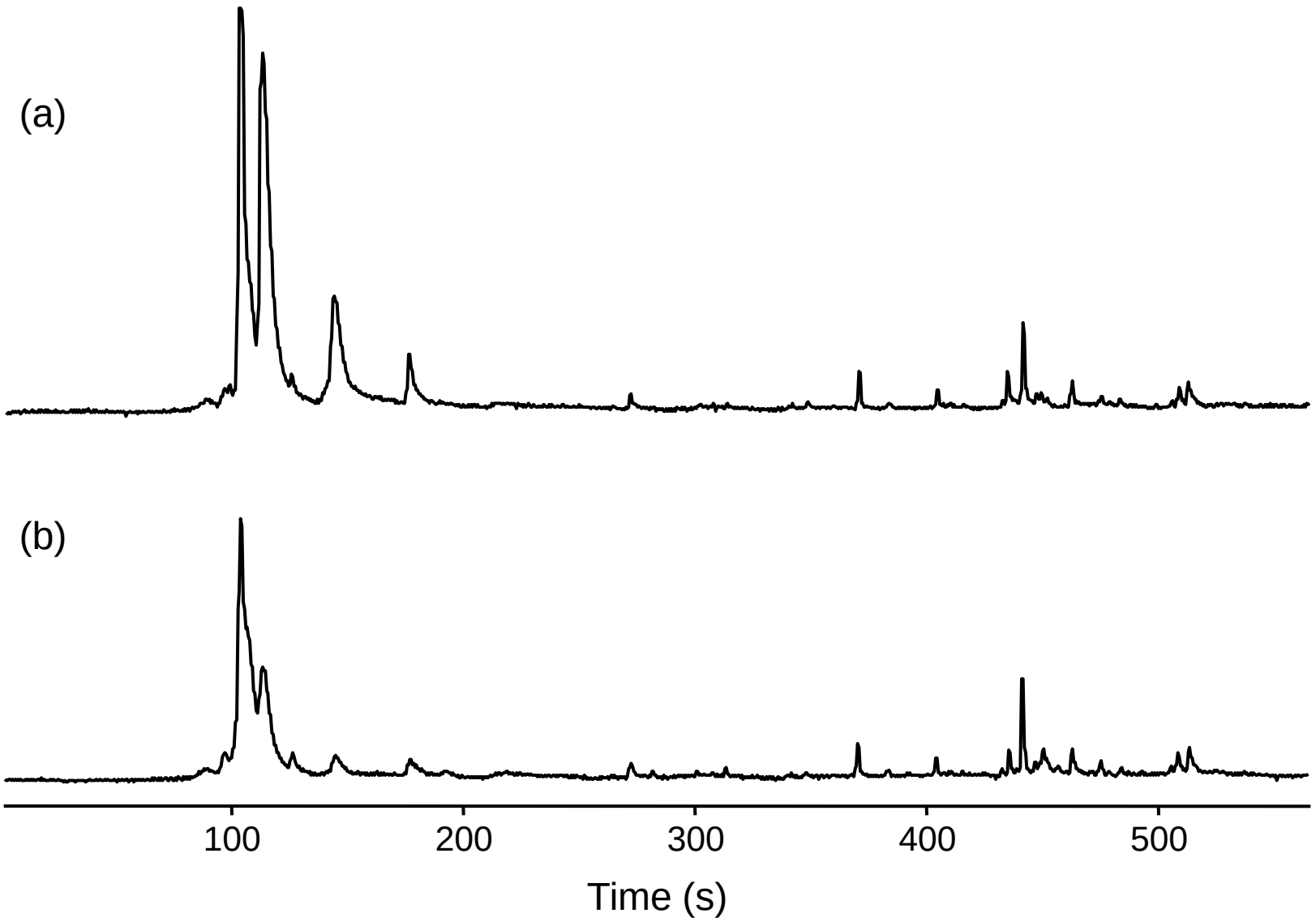


Figure 2



ACCEPTED MANUSCRIPT

- Common containers for sampling collection are compared and discussed
- Advantages and disadvantages of SPME and sorbent trap methods are discussed
- Losses associated to the use of gas sampling bags are discussed
- The use of a new methodology based on needle traps is presented
- A summary is made of the applications in exposure analysis in the past two decades

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