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Chapter

Recent Advances in Targeting Clinical Volatile Organic Compounds (VOC)

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Abstract

This chapter introduces the significance of exploring volatile organic compounds (VOC) in clinical samples. Because exhaled-breath is easy to collect, unlimited, and instruments are already commercially available, VOC analysis in exhaled breath seems to be a promising tool for non-invasive detection of many diseases including infections, respiratory diseases, and cancers. Here, we have focused on some appropriate technologies to extract, pre-concentrate, and evaluate VOC biomarkers in exhaled breath. The second part of this chapter discusses the comprehensive GC × GC in bio-VOCs analysis and illustrates the potential of using this analytical technique.

Keywords: gas chromatography, breather analysis, Sampling, Pre-concentration, VOCs, Biomarker, GC–MS, GCxGC, Mass spectrometry

1. Introduction and scope of the chapter

Inside the human body, cells produce hundreds of biochemical reactions at extremely precise and controlled moments. Cells can be thought of as a factory, regulating what enters or leaves its barrier. From a metabolism point of view, any "error" in biochemical processes (temporary or permanent) leads to abnormal concentrations of metabolites and/or presence of "abnormal" metabolites. Consequently, a wealth of metabolites with low molecular weight as well as high molecular weight can be exploited as a "precious" source of information revealing the metabolic state of the body. Metabolites can be excreted via the urine, feces, saliva, blood, breath, or sweat. Among these metabolites, researchers are actively trying to find biomarkers, identifying the presence of different diseases (cancers, infections and so on). Especially in the instance of different cancers, the lack of specific syndromes with limited understanding of etiology make it difficult to diagnose at an early stage. Nevertheless, biomarkers can turn out to be powerful tool in predicting the development of these cancer and other diseases.

Among all biological samples, exhaled breath has many advantages compared to bio-fluids. First, breath sampling is pain-free, non-invasive, and most important is almost "unlimited". Secondly, breath Volatile Organic Compounds (VOC) are collected from the airways which is directly connected to the entire body via the bloodstream. Blood continuously circulates around body periodically reaching the

air-blood barrier of the alveoli within lungs, VOCs from the whole body can cross this barrier from the blood and be released into the exhaled breath. Conversely, exhaled breath is a very complex matrix and can be challenging to investigate being influenced by a patient's habits, diet, and the environment [1].

The human airways emits a gigantic number (>3000) of VOCs of different origin [2]. VOCs can either be endogenous (arise from the body) or exogenous (environmental source) as shown in **Figure 1(A)**. While it is rather easy to monitor exogenous VOCs, endogenous VOCs can be produced from various sources: normal metabolism of nutrient, inflammatory processes, metabolic processes (diseased and normal), cancerous cells, and microbiome of the oral cavity, airways and gastrointestinal tract. In addition to multiplicity of compounds originating from the different endogenous sources, exhaled breath is saturated with water vapor leading to relative humidity close to 100%, which may impact the collection and analysis of VOCs. In spite of these challenges in exhaled breath research, it continues to attract the interest of scientists worldwide. For the past fifty years, publications on VOCs and exhaled breath have grown exponentially with great effort being devoted to discover VOCs biomarkers related different diseases (**Figure 1(B)**).

A typical workflow for breath collection and analysis in a clinical setting is shown in **Figure 2**. The breath analysis starts with the study design and sample

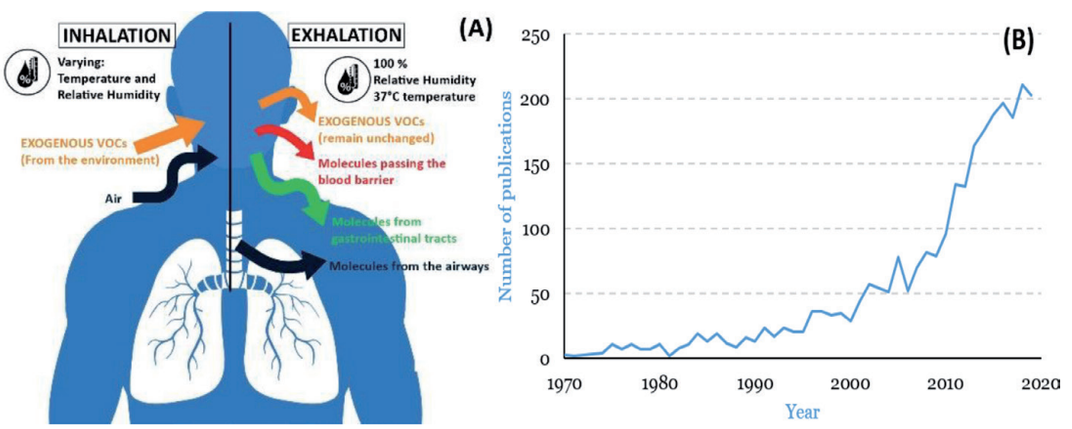


Figure 1.
(A) Pathway of exhaled molecules in the human body, (B) number of research publications involving VOCs and breath field (data from PubMed database).

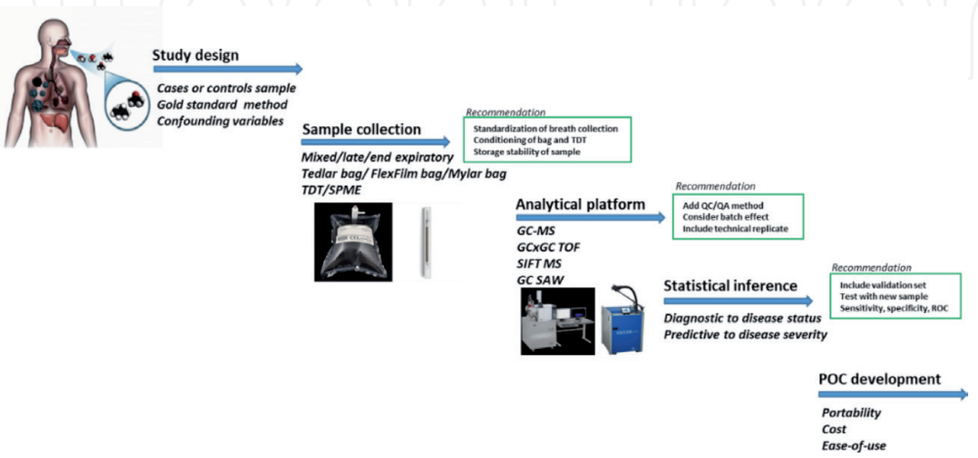


Figure 2.
A schematic of breath analysis pipeline and recommendation. TDT: Thermal desorption tubes; SPME: Solid phase microextraction; SIFT MS: Selected-ion flow-tube mass spectrometry; GC- MS: Gas chromatography–mass spectrometry; GCxGC TOF: Two-dimensional gas chromatography and time-of-flight mass spectrometry; GC SAW: Gas chromatography surface acoustic wave.

collection. Once collected samples must be properly transported and stored until analysis. After the analysis on an analytical instrument important for the specific study design, data would undergo statistical testing and biological interpretation. Finally, results require validation before being directed to the Point-of-Care (POC) development stage.

This book chapter addresses the efforts of many researchers to identify and validate VOCs resulting from various diseases and summarize important technological advancements used to pre-concentrate and analyze VOCs.

2. Capturing, focusing, and storing breath samples

The concept of "off-line" breath analysis can be broadly fragmented into breath sample collection, sample analysis, and data analysis. In this section, a summary of principal methods used to "catch" and to "focus" breath samples is exposed whilst advantages, disadvantages, and practical suggestions are systemically reported.

2.1 Sampling

The major objective of sampling is to take a representative "sample" from a matrix. Usually, a pump or vacuum are used to achieve this process. Decisions to be made must balance between cost including the duration of the sampling period, the size of each sample, and the number of samples.

2.1.1 Bags

Sampling bags are low-cost, whole-air sampling devices for VOCs and permanent gases. Several EPA, NIOSH, and OSHA methods exist for bag sampling for a variety of applications: sources emissions; indoor air quality, workplace atmospheres, and breath analysis [3]. Bags remain popular among researchers, principally due to their low cost and reusability. However, they are known to contain several artifacts, and a tradeoff between competitive pricing and performance is rapidly pointed.

Tedlar® bags are the most commonly used polymer-based bags in air and breath research. Their main disadvantages to the use of Tedlar® bags are largely due to the interaction of air or breath constituents with the polymer which bags are fabricated with. This interaction results in contamination through emission, adsorption, and diffusion. Other polymers were developed to overcome the above-cited disadvantages, such as Mylar (polyethylene terephthalate) and Altef (polyvinylidene difluoride). **Table 1** presents a brief comparison of different type of sampling bags.

Some practical considerations must be taken to avoid sample loos or degradation. Moreover, it is preferable to not reuse bags.

Practical considerations:

- Clean bags before use to minimize the background signal associated with the plastic interior (several times).

- Bag cleaning should be performed as close to the time of sampling (flushing nitrogen for example).

- Analyze or pre-concentrate samples already filled in the bag as fast as possible to avoid losses, interaction with the plastic of the bag, photodegradation, and adsorption.

	Tedlar bags	Multi-layer foil bags	Altef bags
Composition	PVF	4 layers: Nylon, 2 × polyethylene, aluminum foil	PVDF
Advantages	• EPA testing methods • Bag available with stainless steel valves	• Suitable for H_2S • Very low permeability to O_2 and CO_2	• Low VOC background • Lower permeability than Tedlar toward CO_2 and N_2
Limitations	• Background level of phenol and DMAC • High permeation of CO_2 and O_2	• Should analyze within 48 hours especially for CH_4, H_2S, CO, and CO_2	• Less resistance to UV light than Tedlar • Should analyze within 24 hours

Table 1.
DMAC: Dimethylacetamide, PVF: Polyvinyl fluoride, PVDF: Polyvinylidene fluoride.

- Protect the bag from direct sunlight and store it in a rigid container to prevent photodegradation and bag puncture respectively.

- Do not fill the bag more than 80% of its volume

2.1.2 Canisters

Despite their price that is expensive compared to sampling bags, canisters are known to be robust, relatively inert, and non-permeable (example of breath air [4, 5]). They are made with stainless steel and their inner surface interaction with the samples (adsorption, desorption) is of paramount importance especially when lower and lower concentrations exist. The chemical composition of the metal obviously will affect the type of chemical or physical reactions with the sample. To enhance their inertness vis-à-vis sulfur-based samples (for example), canisters are passivated, electropolished, or coated. Canisters are known to be reusable and the sample can be stored until 30 days without loss or degradation.

Practical considerations:

- Canisters are less useful, is some cases, to the storage of semi-volatile and polar compounds DUE to condensation and/or dissolution into water at higher pressure.

- Choose inner surface-deactivated to avoid adsorption and interaction with samples.

- Canisters must be cleaned prior to use. In fact, canisters should be pressurized and evacuated (to be cleaned), and evacuated once more to create vacuum.

An alternative to metal canisters, glass containers have been used for sampling breath [6]. Despite their fragility, they can be more performant than Tedlar bags as reporter by Scott-Thomas et al. [7].

2.1.3 Other containers

Other breath collection container were reported including: gas-tight syringes [8], face mask [9], glass tube [10], and gas bulb [11]. To go further, Lawal et al. [12] investigated breath sampling methods by performing an in depth bibliometric search.

2.2 Focusing

Direct sample introduction by syringe or rotary valves is only suitable for small volumes of "relatively" concentrated samples. In the field of metabolomics, the discovery of diseases biomarkers for example (from urine, feces, breath, tumor and cells) requires analysis of trace and ultra-trace levels of targeted compounds.

Traces analysis methods involve analyte accumulation by sorbent (solid, film) followed by thermal vaporization in the presence of a flow of gas to transport them to the analyzers.

2.2.1 Thermal desorption

Thermal desorption tubes (TD) are the most commonly used medium for the collection and pre-concentration of human breath samples for cancer diagnosis, infections, and bacteria recognition [13, 14]. In thermal desorption technique, sample is swept into the gas chromatograph using heating and a flow of the carrier gas. The desorbed "plug" or "band" of the sample should be as a narrow as possible (chromatographic considerations). However, due to low mass transfer, commonly, sample is first heated slowly letting the desorbed material to be cold-trapped at the head of the column (cold trap or a cryogenic oven). Secondly, the re-condensed sample is then desorbed as the temperature program proceeds.

To trap the VOCs, thermal desorption tubes comprise various sorbent materials (**Table 2**). The sorbent materials can be synthesized (polymer such as Tenax TA) or obtained by graphitizing carbon, which adsorbs a large molecular range of VOCs.

The small size of the tubes (~7 cm length), and their suitability to be used both for active and passive sampling (with or without pump) make them attractive for various applications even standardized methods (EPA—TO-17, ASTM—D6196, NIOSH—2549).

Supelco reported a tool for selecting adsorbent for thermal desorption applications [15]. The goal is to select the "proper" adsorbent that can retains a specific or groups of analytes for a specified sample volume.

Sorbent name	Material	Applications
Tenax TA	2,6-diphenylene-oxide porous polymer	C7-C26
Tenax GR	Mixture between graphite (30%) and (70%) 2,6-diphenylene-oxide	C7-C26
Carbotrap	Graphitized carbon black	
• X		C3-C9
• B		C5-C12
• C		C12-C20
• F		>C20
Carboxen	Carbon molecular sieve	
• 1016		C3-C5
• 10xx		C2-C5
Carbosieve	Carbon molecular sieve	C2-C5
• G, S-II, S-III		

C: Number of carbon atoms.

Table 2.
Various sorbent materials used for thermal desorption applications.

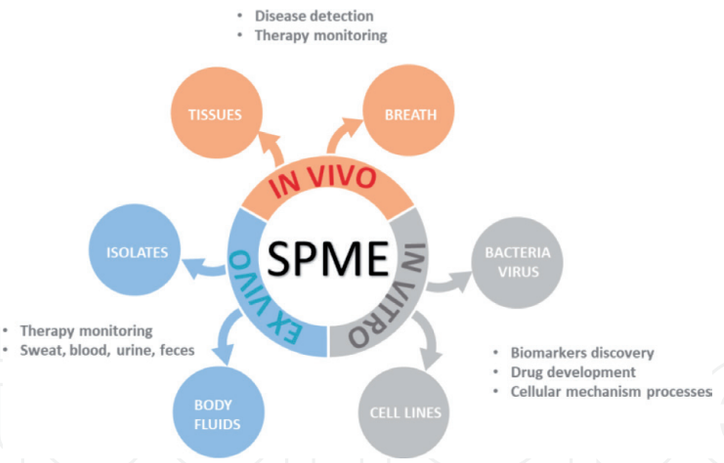


Figure 3.
Common applications of SPM in biology and medical researches.

2.2.2 Solid-phase micro extraction (SPME)

Solid-phase Microextraction (SPME) technic uses a polymer-coated fiber housed in a modified syringe as a sampling device. When SPME is used for analysis, first, the syringe needle is placed into the analyte, and the coated fiber is then exposed. Once the system is brought to equilibrium, the coated fiber is retracted into the syringe needle and removed from the sample (bag filled with breath for example). The needle is then transferred to a heated inlet of the GC, and the analytes are thermally desorbed.

Although SPME is well established, accepted, and validated for various fields (air quality, environment, and food analysis), having yet to gain acceptance as a standard method in biotechnological industries. **Figure 3** highlights exemplary applications of SPME toward health monitoring and biomedical research.

The volatilome of the healthy human body comprises over 1840 VOCs (breath, blood, sweat, urine, and feces) [16]. In this line, SPME-GC–MS was used by Garcia et al. [17] to compare breath issuing from smokers, non-smokers, and patients with laryngeal cancer. Authors have found seven unique VOCs discriminating non-healthy and healthy controls. In the same line, SPME was applied in vitro to demonstrate that particular VOCs are present in exhaled breath of lung cancer patients at significantly different levels than those found in healthy controls [11].

Bean et al. [18], with the help of SPME-GCxGC-TOF-MS, identified 70 compounds indicating presence of *Pseudomonas aeruginosa* bacteria. Similarly, SPME-GC–MS was complemented with SIFT-MS to differentiate 15 clinical strains from 5 environmental strains of *Stenotrophomonas maltophilia* (responsible of lung infection) [19].

Type of coating	Film thickness (μm)	Polarity	Maximum temperature (°C)	Core type
PDMS	30, 100	Non-polar	300	Fused Silica
				Metal
PA	85	Polar	320	Fused Silica
PEG (Carbowax)	60	Polar	250	Metal
Carbopack Z/PDMS	15	Bipolar	340	Metal
PDMS/DVB	65	Bipolar	270	Stableflex
				Metal
Carboxen-PDMS	85	Bipolar	320	Stableflex
				Metal

PDMS: Polydimethylsiloxane, DVB: Divinylbenzene, PA: Polyacrylate, PEG: Polyethylene glycol.

Table 3.
Type of commercially available SPME fiber adapted from Ref. [20].

Type of SPME fiber significantly affect the number and the type of volatile compounds that can be detected, and hence introduces another source of variability to the results [17]. Common type of SPME fibers used to extract compounds are listed in **Table 3**.

PDMS is the coating of choice to extract many classes of non-polar and less polar compounds. At the opposite, polyacrylate coating is the best to extract polar compounds. For example, mixed phase coatings (polar and non-polar) based on PDMS-PEG are used to extract both polar and non-polar compounds.

In addition to the above-cited applications of SPME fibers, their ability to extract semi-volatile or non-volatile compounds was demonstrated [21, 22]. In fact, biofluids including urine, saliva or blood, might comprise many organic compounds which are not present in the vapor phase and hence could not been achieved by headspace analysis [23, 24]. For additional information, a recent review compiling the most recent applications of SPME in biotechnology and clinical studies was written by Filipiak et al. [25].

2.2.3 Other technics

Headspace takes advantage of the closed-vessel equilibrium between either a solid or a liquid and a gas (urine, blood, feces). In the headspace analysis, an aliquot of the equilibrated gas phase is removed from the vessel and GC analyzes the aliquot. This technic is often coupled to SPME (exposing the fiber to the headspace of the sample). Zhang and Raftery reported metabolic profiling of urinary VOCs using SPME-GC–MS [26].

2.3 Sampling breath portions

VOCs present in exhaled breath may also originate from external environment (exogenous VOCs). The goal of sampling breath is to minimize the concentration of exogenous substances. Additionally, dead space air (oral and nasal cavities, gut) acts to dilute and may contaminate VOCs from blood gas exchange. To subtract VOCs levels from contaminant sources, measurement follow specific pathway. Using a capnometer, Miekich et al. [27] found that the end-tidal portion of breath showed the highest concentrations of endogenous and the lowest concentration of exogenous substances. In addition to end-tidal, other terms as Mixed respiratory or late expiratory are found to describe the rest of breath fractions [28].

2.4 Breath sampling apparatus

2.4.1 R-tube

R-tube is well known as exhaled breath condensate portable collector. The sampler includes a cooling sleeve (frozen until needed), a one-way valve, and a plunger. A modified version or R-tube was used by Martin et al. [29] to sample breath volatile compounds by SPME.

2.4.2 Bio-VOC

Bio-VOC is a commercially available system marketed by Markes International. It consists of a hard plastic cylinder connected on one side to a disposable mouthpiece and to sorbent tube on the other side. Poli et al. [30] have used Bio-VOC with SPME fiber to determine aldehydes in exhaled breath of patients with lung cancer.

2.4.3 ReCIVA sampler

Reciva sampler (marketed by Owlstone Medical) is a commercially available sampler. Its uses pressure-modulation (two pumps) breath sampling onto four adsorbent tube. A disposable silicon mask coupled to bacterial filter are also present to prevent cross-contamination (contamination between subjects).

A pressurized clean air supply is however needed to minimize the concentration of exogenous VOCs.

3. Online and offline breath analysis (versus or collaborative)

"On-line analyses" of exhaled breath, sometimes called "direct" or "real-time" breath analyses are mass spectrometry based methods which needs no sample preparation or collection. At the opposite to off-line methods which requires two-step processes (collection and analysis), on-line analyses are one-pot analysis method. The three main online methods for breath analyses are SIFT-MS, PTR-MS, and SESI-MS.

SIFT-MS is more suitable for targeted analyses however their limiting factor is low mass resolution. Recently, new enhancement is introduced to this method including the use of time-of-flight (TOF) mass analyzers and the use of the electric fields in the drift tube (SIFDT).

PTR-MS had been first established in the field of environmental analyses and recently in the field of breath analyses. Its limitation is set by the principle that only VOCs having a higher proton affinity than that of water can be detected. New enhancement including the use of TOF and more recently a quadrupole boosts this method to detect higher than m/Δm 10 000.

SESI-MS has been found to be the most sensitive for polar compounds reaching sub ppqv. The use of SESI-MS in analyses of the breath condensate is well established by high-performance gas chromatography (HPLC). However, the main disadvantage of this method is that use to analyze gas phase is not yet possible.

Undoubtedly, Mass Spectrometric methods are the most sensitive and the most appropriate for compounds discovery and identification. However, their use in clinic studies remain limited due to practical considerations. Bruderer et al. [31] reviewed extensively on-line methods for exhaled breath analyses.

4. Two-dimensional GC

In the early 90's, two-dimensional gas chromatography has become available for the separation and the identification of complex mixtures. This section demonstrates the versatility and applicability of 2D-GC and an analytical tool for breath analyses.

4.1 Principle

In GC, higher the resolving power, the better the performance. It is understandable that, if the column becomes longer, they could resolve more analyte. There are multiple examples of a very long (>100 m) column used on a very complex sample separation in a 1D GC experiment [32]. Other than the length of the column, there are few more approaches have been taken to improve

the resolving power of the samples. One, forwarding a part of the sample to a second column to be better resolved based on another separation mechanism. This process is known as the Classical Multidimensional Gas Chromatography or MDGC [33]. The second approach transfers effluent of the first column to a second column for a comprehensive separation of the samples by a modulation device that forwards a very narrow band from the first to the second column. This process is named as GC × GC or Comprehensive two-dimensional gas chromatography [34].

The GC × GC system is operated as two different stationary phases that have different separation mechanisms are connected to each other so result in an orthogonal separation of the sample [34]. But to achieve this, there is a special device called "modulator" is connected in between the two phases, so it can sample and re-inject the first column effluent to the second phase for a complete independent separation [35].

The heart of GC × GC is the modulator. There are extensive reviews that already exist on the fundamental of the GC × GC modulator [36, 37]. There are different types of modulators that have been introduced in the field, such as thermal modulator, valve-based modulator and, flow modulator. The main task of all these modulators are to sample the primary column effluents to the second column (a sharp pulse of 1D peak). One main difference is the requirement of the focusing steps. The cryogen-free modulator such as the flow modulator, Peltier modulator does not have any focusing effect where the cryogenic modulator like longitudinally modulated cryogenic system (LMCS) has focusing steps, hence requires liquid N_2 or CO_2 to cold trap the analyte from 1D before eluting to the 2D column. Although it is a compensation of the peak capacity, the consumable-free modulator is getting more popular due to low set-up fees, less consumable and maintenance free and lowest safety issue. **Figure 4** gives an example of 2D-GC configuration.

This is vital for the detector of GC × GC to have certain characteristics. This is because the second dimension separation occurs quickly, demanding a fast acquisition detector with at-least 50 scans/second. The Flame Ionization Detector (FID), Electron capture detector (ECD) or Flame photometric detector (FPD) also acquire fast data and are able to collect enough data points for even 300 ms width GC × GC peak. The high-speed MS detector time of flight (TOF) is often used as a detector for the GC × GC. Using MS with GC × GC separation

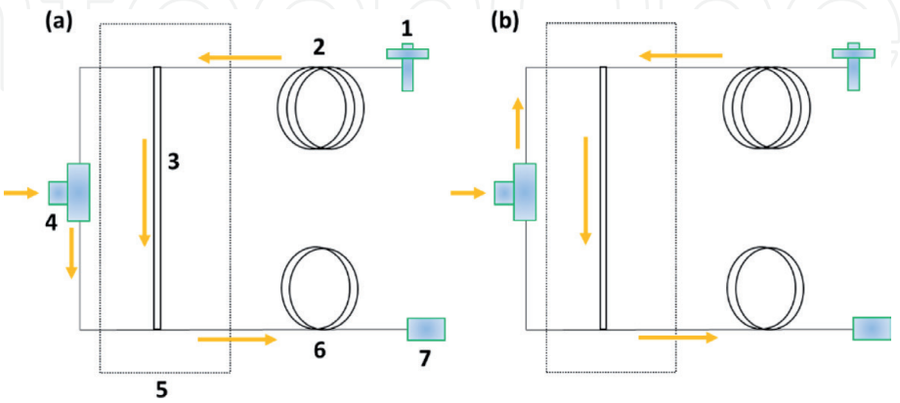


Figure 4.
The capillary flow technology (CFT) based flow modulator design. The arrows show the flow direction. The injector (1) is connected to the primary column (2) that passes through a channel (3) that is mounted inside of CFT device (5). A modulation valve (4) is connected to supply ancillary flow of carrier gas supply to divert the flow in a downward direction as seen in (a): Load position, or upward as in (b): Inject position toward to a 2D column (6) to detector (7). With permission from [35].

adds massive benefits for compound identification through the deconvolution of the mass/charge ratio (m/z) and results presented in a two dimensional contour plot. There are few software package available to do this deconvolution and identification of compounds with spectral matching to known compound libraries.

The primary benefit of using GC × GC for VOCs analysis is the enhanced selectivity that helps complete separation of the complex VOCs matrix. A number of literature has been reported to utilize the GC × GC for this purpose, and discussed more detail in the next section. The typical column configuration that are used with GC × GC for the breath analysis are a non-polar long column (30 to 60 meters) such as (5%-phenyl)-methylpolysiloxane phase as first dimension and a short (1–2 meters) polar column such as Wax phase as second dimension [13, 14, 18]. An interesting optimization study also recommend the same non-polar and polar column set for VOCs analysis [38]. The study compares a number of commercially available column such as 1%-phenyl, 5%-phenyl, and WAX phase as ^{1}D column and WAX, 5%-phenyl and ionic liquid column as ^{2}D phase. Based on the observed chromatogram and tradeoff between column maintenance and separation efficiency, the orthogonal non-polar and polar set was recommended.

Nevertheless, the 1D GC is long been used for the VOCs analysis as they are the most robust and reliable tools for the VOC analysis and available in many labs around the world. The benefits of using 1D classical GC for VOCs include the reproducibility, simple operation, presence of the comprehensive library and ease of data management. There are a number of mass analyzer technologies such as quadrupole, TOF and Ion trap that also makes 1D GC as a strong tool for the VOCs analysis. The 1D GC–MS systems is reported to analyze VOC "propofol", a drug that is used as anesthesia during surgery [39]. These results indicated a precise and sensitive determination of propofol in breath and blood by the GC–MS analysis. Ulanowska et al., determined the VOCs from the *Helicobacter pylori* as an indicator of gastrointestinal infection [40]. Their results identified a panel of breath metabolites, isobutane, 2-butanone and ethyl acetate as potential biomarker of the *H. pylori* infection.

4.2 Applications

Breath analysis is traditionally used for the signature of the disease. There is a disease-specific chemical signature that can be differentiated based on the breath

Common breath biomarkers between Human and Monkey (total match is 40.8%)		
MW (g/mol)	Human	Monkey
Exact match (10.5%)		
114.2	Heptanal	Heptanal*
170.3	Dodecane	Dodecane
184.4	Tridecane	Tridecane
168.3	hexylcyclohexane	Hexylcyclohexane
Isomer match (30.3%)		
136.2	l-beta-pinene	Trans-á-Ocimene
	camphene	beta-phellandrene
142.3	Heptane, 3-ethyl-2-methyl-	2,3,6-trimethylheptane
	Octane, 2,6-dimethyl-	-
	5-ethyl-2-methylheptane	-
170.3	3,7-dimethyl-decane	2,5-dimethyldecane
170.3	4-ethyl-5-methyl-nonane	2,2,4,6,6-pentamethylheptane
184.4	4-methyl-dodecane	4-ethyl-2,2,6,6-tetramethylheptane
120.2	1,3,5-trimethyl-benzene	1,2,3-trimethylbenzene
226.4	Pentadecane, 5-methyl-	Hexadecane
128.3	Heptane, 2,4-dimethyl-	2,2,3-trimethylhexane
	Nonane	-
	2,3-dimethyl-heptane	-
168.3	4,6,8-trimethyl-1-nonene	-

* confirmed by authentic standard

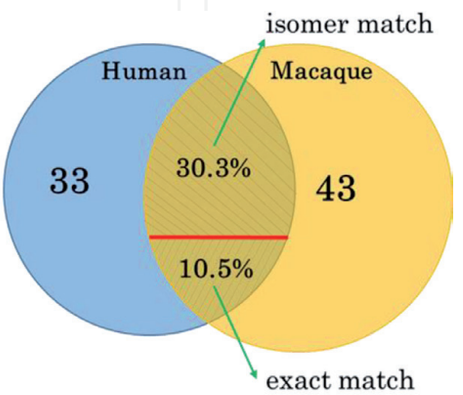


Figure 5.
The VOCs from the NHP and humane identified by similar GC × GC TOF MS. The VOCs signature compounds from the different pathway (prepared from the data published on [14, 39]).

sample. Numerous example has been published where the breath samples were analyzed and compared with the healthy person breath to determine the VOCs that express differently from the diseased person. A good example is Tuberculosis, this is a disease caused by a bacteria called "*Mycobacterium tuberculosis*". The main residing location of these bacteria is in the lung when they interact with the lung cell and use the energy pathway to proliferate. Hill group demonstrates the VOCs from the culture of *Mtb* are different than the VOCs from the empty culture using the GC × GC TOF instrument [41]. Using a similar GC × GC method, they have also found a difference between Mtb infected Non-Human Primate (NHP) breath from uninfected breath [13, 42, 43]. The match between the documented VOCs is listed below. The heptanal, dodecane, tridecane, and hexylcyclohexane were detected from both NHP and humane infected breath VOCs. **Figure 5** shows the match between the VOCs detected from human and NHP. Using a similar instrument platform, almost 50% of VOCs could be matched between the two species' breath.

In addition to breath, GC × GC TOF MS has become an essential tool for metabolomics. This process tries to look at the volatile, semi-volatile, and heavy boiler compounds from the biological fluid such as serum or plasma, cell, urine, and other biofluids [44]. Mishra et al. reported an interesting comparison study between the GC × GC and 1D GC for the serum sample. According to the author, the GC × GC can detect about 5,000 metabolites whereas the 1D GC could only detect about 500 metabolites. It might seems overwhelming as the 1D GC was equipped with high-resolution Orbitrap MS which is high resolution but less sensitive than the QTOF used with the GC × GC system [45]. Yu et al. optimized the GC × GC parameters to identify the maximum number of metabolites including the VOCs from the biofluids [46]. Urinary aromatic amine is an indicator of cigarette smoking. SPME of urine volatiles of smokers and analysis by GC × GC MS has revealed more than 150 aromatic amine compounds which is a much higher number from the previous 1D GC analysis [47]. VOCs analysis of biological fluid using the GC × GC techniques is still raising and within the next couple of years, this process will become a more prominent and established process for the VOC analysis.

4.3 Comparison with other analytical methods

There are several methods currently utilized by dozens of research groups to analyses the breath VOCs. The essential question here to ask which type of method might be a stronger candidate for the breath volatiles. There are many well-documented reviews summarized the analytical methods used for the breath VOCs analysis [48–53]. Essentially the basic method for analysis breath biomarkers is classified based on the objective of the study. For instance, the chromatography separation and mass spectroscopy for the purpose of identification (untargeted) or quantification (targeted) analysis. A comparison table between the instrumental platform used for the VOCs analysis is provided in **Table 4** with the advantages and disadvantages of each method. The critical comparison of these techniques is not intended to describe here but it can easily see the high solving power of the GC × GC method put it as the highest sensitive device currently available for the VOCs analysis. To sum up this discussion, all analytical platforms used for VOCs analysis could be classified into few subcategories (a) sensor array for detecting any specific analytes (b) separating a mixture of analytes from the matrix, and (c) the high-resolution mass spectroscopy for identification. Reader are referred to Sethi et al. [54] for more detail comparison.

Intrumental platform	Advantages	Disadvantages
Comprehensive two dimentional GC × GC	High sensetive identification Reproducible quatification Maximum number of VOCs	Required special tools and software
Gas chromatography with mass spectroscopy	Sensitive at ppb level Useful to identify unknown Robust and reproducible	High temperature operation Known standard requires Not cost effective
Ion mobility spectrometry	Sensitive at ppm level Portable Cost effective	Chemical fingerprint and identification are not possible Offline analysis Low sensitive
Selected ion flow tube mass spectrometry	Sensitive at ppb level Rapid and cost effective Portable and online	Chemical fingerprint and identification are not possible
Proton transfer spectrometry	Sensitive at ppb level Direct injection of VOCs Online measurement and monitoring	VOC chemical identification and complete profiling not possible
Various chemical sensor matrix platforms/e-noses	Sensitive at ppb level Cost effective and portable Fast and easy Point of care compatible	VOC chemical identification and complete profiling not possible

Table 4.
Advantages and disadvantages of some of the methods currently used for VOC analysis in clinical matrices.

5. Conclusion

Clinical VOCs may have potential for noninvasive pathological diagnosis. However, discovering biomarkers associated with specific disease requires standardization methods for both sampling and analyzing. The present contribution discusses recent advances in analyses of exhaled breath VOCs and focuses on chromatographic technics for off-line analyses. Mass spectrometric technics for on-line analyses were illustrated and their potential for VOCs discovering were demonstrated. The last part of this chapter discussed the comprehensive GC × GC technic and its ability in bio-VOCs analyzing. This technic has been proven as effective due to enhanced peak capacity and sensitivity.

There are several research centers working on the two competitive technics (on-line and off-line) and significant amount of resources are dedicated. We believe that breath analyses will become the method of choice for diagnosis of several pathologies such as Asthma and other infections. As discussed above, standardization is crucial to go further in the process of validation with a sufficient cohort size.

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