Breath analysis is an emerging scientific field with promising medical applications. Volatile organic compounds (VOCs) of exhaled air are targeted and analysed with the use of various analytical instruments including GC-MS, PTR-MS, SIFT-MS, IMS, spectroscopic techniques, e-nose and sensors; potential differences in the emitted concentrations of specific or unspecific (pattern of compounds) biomarker(s) are related to health status or disease. Human breath is a clean, inexhaustible and non-invasive source of endogenous information; this makes it ideal for sampling especially vulnerable people and children. Despite its numerous and wide promising applications, it remains in an infant stage, as it suffers from standardised practices, presenting several qualitative and quantitative pitfalls. The sample, the size, the chemical diversity of exhaled breath volatiles, the role of confounders, the various sampling methodologies and techniques, sample treatment and data interpretation are considered among others, important factors of uncertainties and need of special attention. In this context, an overview of the critical issues explored in breath analysis are highlighted and reviewed, towards the vision of development personal care hand-held monitoring devices. The understanding of these manifold issues assists researchers to validate their experimental design, towards adopting standard practices in the field of breath gas analysis. The ultimate goal, is sometime in the near future, breath analysis to become a reliable tool in the clinical setting, both for medical experts and human individuals for early diagnosis, clinical and personal monitoring.

1. Introduction

The last decade, there has been an increased interest on the determination of Volatile Organic Compounds (VOCs) for a number of challenging and novel applications, in the areas of:

- a) Bio-analysis (disease prognosis and monitoring) [1]
- b) Environment (indoor/outdoor/atmospheric pollution, plant/soil/waste emissions, etc.) [2, 3]
- c) Forensic and security (Urban Search and Rescue (USAR) applications) [4]

The common core of these applications is the qualitative and quantitative determination of VOCs. They are defined as a large group of anthropogenic (xenobiotic) or biogenic organic compounds with relatively high vapor pressures. VOCs are emitted by all living organisms and can also be found in numerous domestic/industrial products. They can be potentially malodorous and hazardous for both human health (e.g. eye/nose irritation, headaches, nausea) and the environment (air, water, soil). The level of effect on humans and the environment varies greatly, depending on the substance, the amount and time of exposure, necessitating the identification and quantification of VOCs. The emission of VOCs creates a volatile chemical signature characterizing the source; this may lead to the detection of human presence and even in the noninvasive identification of human/plant diseases (e.g. microorganisms, infections).

2. The Human “Volatileome”

The study of the human volatileome includes the determination of VOCs released by body tissues and biological tissues, as presented in Figure 1. Their concentration is changing in pathological conditions enabling their use as novel medical diagnostic tools for various types of cancers, oxidative stress, asthma, diabetes, kidney-, liver- failure and many other medical disorders.

3. “Breathomics”

The early observations of Hippocrates, were confirmed with the analytical findings of Nobel Laureate Linus Pauling (1971) and therefore research on breath analysis (“breathomics”) has been boosted; in parallel headspace analysis on the rest biological tissues followed. VOCs offer unique insights into ongoing biochemical processes in healthy and diseased humans [1]. Samples are non-invasively obtainable, as often as desired with acceptable discomfort, even during human daily activities such as sleep, exercise, etc. It should be noted, that sampling procedure and methodology has been expanded with success to mammals, despite their size (e.g. mice, cow, dolphin). Nevertheless, the big challenge, remains that of development point of care medical diagnostics devices for personal monitoring.

4. Analytical Challenges

- Sample diversity
- Disease
- Analytical technique → Inter-comparison
- Unspecified (common) biomarker

5. Uncertainties

According to the Sampling and Standardization Interest Group of IABR [7], concentrations of VOCs in breath research are affected by the following factors [5-7]:

- Breath sampling (e.g. sampling conditions, part of exhalation sampled, direct sample VS sampling in bags/containers, traps, storage, handling etc.)
- Storage/transportation of breath samples (e.g. bags, containers, transfer lines, traps)
- Pre-concentration/transfer of breath samples
- Applied analytical method (e.g. selectivity and sensitivity of the method)
- Physiology (e.g. hemodynamic effects, breathing pattern, paced breathing, breathing flow distribution)
- Acute and previous exposure
- Nutrition, medication
- Pathology
- Genotype/phenotype, age and gender
- Effects/emissions of microorganisms
- Physicochemical properties of the VOC (in-out distribution)
- Identification of potential VOC biomarkers (features, sum parameters, tentative, reference substance)
- Quantitative assessment (rel. counts/areas vs. external calibration with reference substances)
- Units of VOC concentrations (nmol/L, nmol/m³, BS, nmol/EICO₃)
- Data analysis/interpretation (e.g. in studies with a large number of potential VOC markers).


- Although hundreds of VOCs have been described as potential biomarkers for diseases or disease states, however they have never been confirmed by independent studies and are therefore not viable in the clinical setting.
- There are not “unique” or “exclusive” VOC breath markers for certain diseases.
- The concentration changes of potential breath biomarkers may indicate pathological changes but may also reflect physiological effects, confounding variables (e.g. previous or acute exposure, medication, nutrition, microorganisms), methodological effects (sampling, storage, analytical set-up) and genetic/phenotype predisposition.

References:

5. Beauchamp J., Current sampling and analysis techniques in breath research-results of a task force poll, J. Breath Res. 7 (2013) 047107.
7. Sampling and Standardization Interest Group, International association of Breath Research.