Real-time analysis of VOCs in human breath, using SIFT-MS

Monitoring of the numerous volatile compounds in breath is a promising and expanding field. While techniques such as GC-MS have provided highly sensitive analysis of the compounds in breath, slow rates of sample throughput have limited development of breath tests for research and diagnostic purposes.

More recently, real-time mass spectrometry techniques, such as SIFT-MS, have become available. The ability of SIFT-MS to analyze complex, humid breath mixtures in real time has overcome previous limitations, allowing the exhalation physiology of multiple compounds to be studied simultaneously as each breath is exhaled.

This white paper provides an overview of technologies used in breath analysis, then discusses relevant characteristics of SIFT-MS and how they have been applied to actual breath analysis studies.

Introduction

Exhaled breath contains an abundance of compounds that can be used to study and diagnose health issues. Alterations to the concentrations and composition of volatile compounds on breath can indicate underlying changes to biochemistry, colonization by microbes, or exposure from exogenous sources. Importantly, collecting of breath samples is noninvasive, simple to perform, agreeable to patients, and fast.

The first step toward routine breath-based diagnostics involves fundamental research—a key component of which is identifying and utilizing the best available breath analysis technology. The development of assays for breath volatiles has traditionally been hampered by a lack of appropriate analytical devices that are capable of reliably detecting the low concentrations of volatile organic compounds (VOCs) in breath.

In this white paper we overview some of the common research and clinical scenarios where quick, reproducible analysis of volatile compounds is of benefit, and compare commercially available detection technologies, including Selected Ion Flow Tube Mass Spectrometry (SIFT-MS).
**Commercially available breath research technologies**

The ideal analytical instrument for breath research would be highly sensitive and able to detect and quantify compounds or patterns of compounds in humid gas mixtures. Several commercially available technologies are compared in Table 1.

**Table 1.** A comparison of the characteristics of a variety of commercially available breath research technologies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nitric oxide analysers</th>
<th>Sensors</th>
<th>IMS</th>
<th>GC-MS</th>
<th>PTR-MS</th>
<th>SIFT-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Breadth of Analysis</td>
<td>Low</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Specificity</td>
<td>Low</td>
<td>High</td>
<td>Medium</td>
<td>Very high</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>Very high</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Speed</td>
<td>Real time</td>
<td>Real time</td>
<td>Real time</td>
<td>Off line</td>
<td>Real time</td>
<td>Real time</td>
</tr>
<tr>
<td>Required user skill level</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Low&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Consumable costs per sample</td>
<td>Low-Medium</td>
<td>Low</td>
<td>Low</td>
<td>Medium</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Low-Medium</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
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<tr>
<td>Sample preparation</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Pre-concentration</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Adaptability</td>
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<td>None</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Biological safety</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Offline sampling</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. With preconcentration.
2. For routine operation using preloaded methods.

Nitric oxide analyzers and other VOC sensors generally only monitor single compounds and so have limited use in breath research, where the ability to measure several chemically distinct gases in each exhalation is advantageous.

Traditionally techniques that combine chromatography with mass spectrometry have been used for breath gas analysis. Of these, gas chromatography mass spectrometry (GC-MS) has been the most widely used to date. GC-MS is generally viewed as the leading technology for VOC analysis, because it fulfills the essential requirements of compound selectivity and limit of quantitation. However, since GC-MS is not a real-time technique (analyses typically take 20 to 40 minutes), its strength is in off-line identification of marker compounds rather than in routine breath analysis. Another key limitation of GC-MS in breath applications is its requirement for sample pre-concentration (via techniques such as thermal desorption) both to concentrate breath VOCs and to remove humidity, which it cannot handle.

Mass spectrometry techniques that analyze samples in real time and without pre-concentration offer significant advantages to breath research. The two leading real-time methods are SIFT-MS and proton transfer reaction mass spectrometry (PTR-MS). These methods eliminate chromatography and utilize soft chemical ionization to simplify compound mass spectra. Of these two, SIFT-MS is unique because multiple reagent ions can be applied during the analysis of a single breath, due to low
millisecond switching times between reagents. This markedly increases selectivity over techniques that use only a single reagent ion or can only switch on a timescale of seconds, such as PTR-MS. Therefore only SIFT-MS offers highly selective real-time mass spectrometry analysis of high-humidity breath samples.

**Breath research scenarios**

1. **Single breath resolution**

A key strength of real-time analysis technology is its ability to capture multiple concentration measurements in each breath exhalation. This allows the construction of whole breath profiles, rather than single-point concentrations (as is the case with GC-MS). Profiling of each breath allows the physiological and biological origin of each compound to be investigated. SIFT-MS, with its rapid response time and low sample volume requirement, can capture uniquely detailed breath profiles, as illustrated in Figures 1 and 2.

**Figure 1.** Three exhalations directly into a SIFT-MS instrument, showing single breath resolution of Acetone (grey) and Isoprene (black).

![Figure 1](image1.png)

**Figure 2.** Single breath SIFT-MS analysis for hydrogen cyanide (grey) and hydrogen sulfide (black).

![Figure 2](image2.png)
Effective breath by breath analysis requires high-sensitivity analysis without sample preconcentration or other time consuming sample handling procedures. This significantly limits the effectiveness of GC-based techniques for breath analysis, as they are not real time and require preconcentration to achieve sufficient sensitivity for this application. Additionally, neither GC, IMS or PTR-MS techniques can analyze multiple chemically diverse compounds in a single analysis, which SIFT-MS can do due to its rapid switching of three reagent ions.

2. Non-invasive sampling

A significant advantage of breath testing is the non-invasive nature of its sample collection. Non-invasive sampling means breath testing is:

- Better tolerated by patients and study participants
- Able to be performed continuously or repeatedly
- Generally quicker and less expensive than traditional sampling methods such as repeat blood sampling.

Continuous breath monitoring in real time allows monitoring of multiple compounds for pharmacokinetic, therapeutic (Figure 3) or occupational exposure (Figure 4). The subsequent rapid reporting of breath analyte concentrations and trends at point of care has the potential to guide timely therapies.

**Figure 3.** Simultaneous SIFT-MS monitoring of ammonia (diamonds), acetone (squares) and trimethylamine (right hand axis; triangle) in breath every 30 minutes whilst a patient was undergoing dialysis.
Figure 4. Decay of mesitylene after exposure, measured by SIFT-MS in a volunteer’s breath after a two-hour controlled exposure.

3. Population testing or screening

Practical screening of large populations via breath testing requires a technique that offers non-invasive sample collection, fast throughput, real-time analysis, and low operating and consumable costs. All of these features are inherent characteristics of SIFT-MS, which makes rapid high-sensitivity population screening feasible.

In a 2007 pilot study, SIFT-MS was used to quickly measure analyte concentrations on the breath of more than 200 children aged 5 to 13 years. Each child provided a 10-second breath exhalation for analysis of seven VOCs. A biological filter on the sample inlet ensured participants were protected from cross-contamination. Figure 5 illustrates the study’s results, while Figure 6 illustrates throughput rate from a smaller screening study.

Figure 5. Distribution of breath isoprene concentrations, measured by SIFT-MS in 230 children aged 5 to 13 years.
4. Offline sampling

Analysis of samples stored in containers such as canisters and sample bags adds a useful dimension to breath testing, especially when patients or research subjects cannot be directly accessed, or where comparative analysis of real time and stored samples is beneficial.

Well-designed SIFT-MS instruments include the sample inlet flexibility and software interface required to quickly switch between sample inlet mechanisms, allowing online and offline analysis of breath samples, with minimal delay between samples. Sensor devices and GC-MS are not as suitable for changing between online and offline sampling methods.

Conclusion

Mass spectrometry techniques such as GC-MS have made a valuable contribution to breath-based research. Recently, real-time mass spectrometry techniques such as SIFT-MS and PTR-MS have promised to extend this contribution by delivering breath-by-breath instantaneous analysis.

SIFT-MS has now demonstrated a practical system for real-time breath analysis. With its unique ability to analyze complex and high-humidity samples, in real time and with high specificity, SIFT-MS has produced accurate and reliable multi-compound breath profiles as the breath is exhaled.

SIFT-MS therefore opens a variety of opportunities for breath-based research, including areas such as rapid, non-invasive screening of large populations, and timely monitoring of changes in patients’ breath profiles. These features are enhanced further by the low operating costs and relatively simple operating characteristics of SIFT-MS.

Real time, highly sensitive breath analysis is now a reality.