SICRIT®-HRMS for Exhaled Breath Analysis

Summary

We show, how easily the SICRIT® Ion source can be adapted to high-resolution MS instruments for trace analysis of biomarkes and metabolites in exhaled breath.

Introduction

The analysis of exhaled breath and its condensate, respectively, is a field of modern clinical medicine that focusses on identification and quantification of volatile organic (VOC) biomarkers for non-invasive medical diagnosis. For example, different biomarkers related to oxidative stress can be found and associated to pulmonary and inflammatory diseases like asthma or chronic obstructive pulmonary disease (COPD).[1]. Also, research on volatiles linked to lung cancer is a promising application for breath analysis. However, VOC detection in breath is not only limited to medical diagnosis, also information for food-related issues can be gained. E.g., metabolism and reaction pathways of food aroma compounds can be illucidated.

Of course, depth and quality of information in breath analysis is strongly depending on the VOC sampling and detection method used.

Since mass spectrometry is one of the most selective and sensitive techniques for biomarker identification, MS-based methods are dedicated for these purposes. There are some approaches in VOC breath analysis taking advantage of MS, for example SESI (secondary electro-spray ionization)-HRMS and PTR-(proton-transfer-reaction)-ToF (time-of-flight)-MS.[2,3] With the SICRIT® Ionization technology, we present a flexible and powerful alternative allowing for real-time HRMS monitoring of breath, that exhibits several beneficial features, as following:

- Soft ionization with broad polarity range
- High-sensitive flow-through ionization
- High-resolution MS data for molecule identification
- Plug & Play installation on any LC-MS within minutes
- Flexible coupling to chromatography, autosampler, etc.

Experimental Setup

The SICRIT® ionization source is interfaced with the atmospheric pressure inlet of a high-resolution mass spectrometer (Thermo Fisher LTQ Orbitrap XL), which is constantly drawing air through the source.

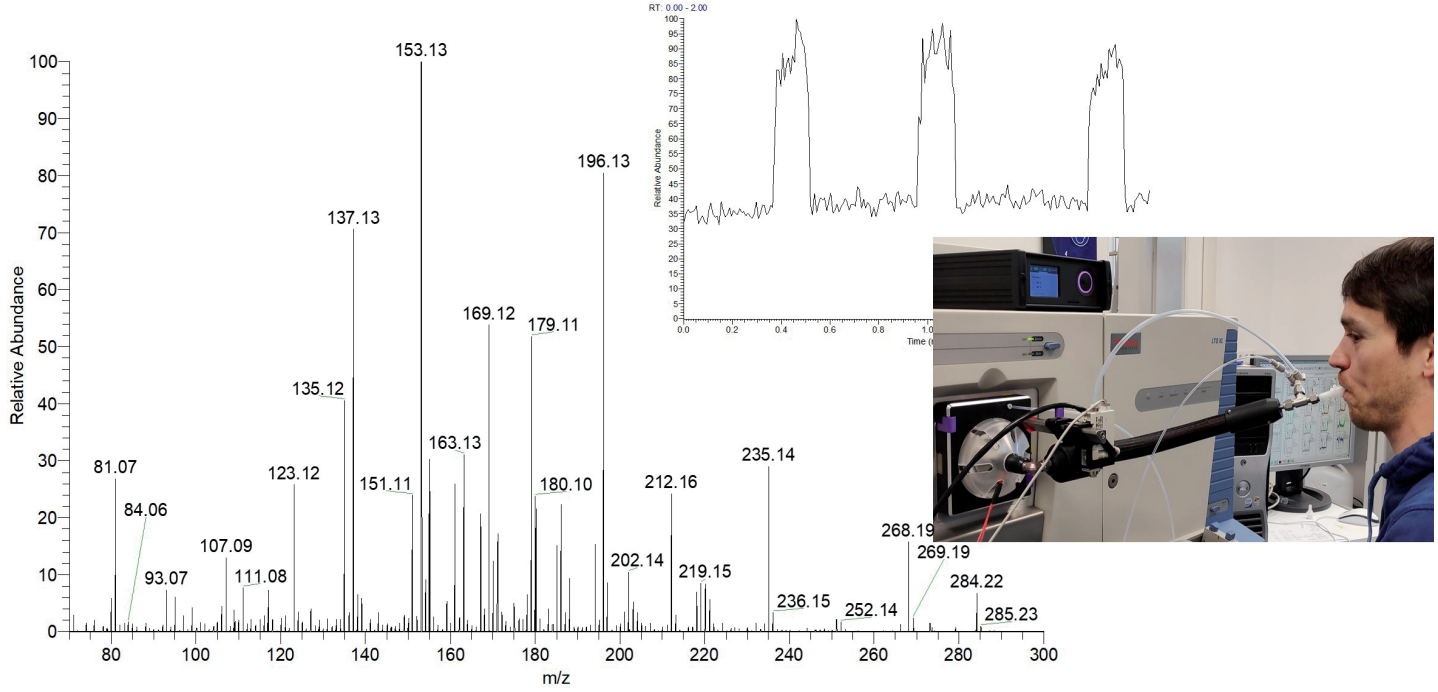

Figure 2 - representative averaged full HRMS spectrum (70-300 m/z) of one exahalation, obtained in postive mode on LTQ Orbitrap XL system (Thermo Fisher).

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In our tests, we used a home-built breath analysis interface for transfer ot the exhaled breath into the source. The setup consisted of a deacticated solid steel tube encased in a heated hose connected to the ion source. To avoid condensation, the transfer tube was heated to 150°C. The exhaled air flow was kept at 7.5 L/min under a nitrogen overflow introduced via a Swagelok T-piece. For breath sampling, disposable mouth pieces with reverse breathing lock were used.
MS detection was performed in full-scan positive mode with a resolution of 30,000 FWHM (mass range 50-300 m/z) and spectra were evaluated using a 5 ppm window.
For analysis, three consecutive exhalations of the test persond were recorded and saved in one data file.

Results

Motivation of our proof-of-concept study was to examine the potential of SICRIT® technology to be used for real time exhaled breath analysis.
First of all it should be mentioned, that we did our tests with healthy test persons, hence it couldn‘t be expected to identify biomarkers only present in breath of patients with certain clinical symptoms. Nevertheless, the feasibility of the setup and the ionization efficiency could be proved by spectrum analysis regarding ubiquitous compounds found in breath which are associated with food and diet.

In Figure 1, an averaged full spectrum of one single exhalation is shown. The feasibiltiy and reproducibility of the measurement setup can be clearly verified by the TIC signal, where each breath results in significant signals increase over the whole exhalation time.
Most ion species are in the mass range between 100-200 m/z as it could be expected for VOCs in breath. Also, some small volatiles with m/z < 100 can be found in the spectra and identified.
Looking into detail, several volatile biomarkers, which are associated with human health status, could be clearly assigned by their exact mass. Acetone is one of the most cited biomarker in breath as it is not only one common ketone body causing halitosis, but also the main indicator for diabetic ketoacidosis. Other small VOCs like urea, pyridine and indol also could be identified (see Figure 2), showing the capability of SICRIT® for diagnostics related to general health conditions.
Another molecule class which is an actual topic in medical breath analyis are aldehydes. As reported by Singh et al., 2-alkenals, 4-hydroxy-2-alkenals, and 4-hydroxy-2,6-alkendienals are hypothesized markers for oxidative stress

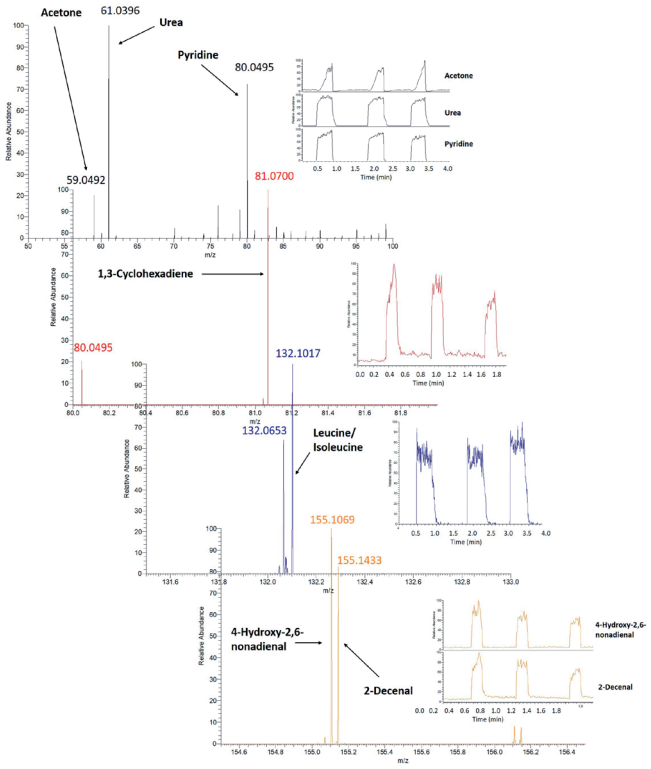

Figure 2 - Identified biomarkers in exhaled breath, assigned by exact mass.

due to chronic inflammatories like COPD. [2]
As shown in Fig. 2, we could identify representative aldehydes as well as some amino acids like leucine/isoleucine, proline, and valine, which are very relevant for diverse diagnostic metabolite tests. This underlines the potential of SICRIT® soft ionization for comprehensive non-invasive biomarker analysis.

Conclusions

MS-based breath analysis is one of the youngest research areas in biomedical diagnostics. For patients, the non-invasive testing is very comfortable and diagnostic findings can be obtained in real-time. The analytical power of HRMS for metabolite and biomarker identifcation is unquestioned, however, the plug&play design of SICRIT® now drastically facilitates the interfacing setup with the MS-instrument. Furthermore, easy combination of the SICRIT® Ion Source with chromatography or liquid injection is possible. Thus, complementary analysis of other biological matrix on the same MS may result in unique data for wide-ranging research and diagnostic tasks.

References

[1] A. Amann et al., J. Breath Res. 2014, 8 (3):034001
[2] K.D. Singh al., J. Breath Res. 2018, 12, 027113
[3] G. Pugliese et al., Analyst 2019, 144, 7359-7367.

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