SUPER SESI

APPLICATION NOTE

(This note describes how the chemical composition of breath can be analyzed in real time with High Resolution Mass Spectrometry and SUPER SESI).

Exhalion (left), SUPER SESI ion source (right)

Breath Analysis in real time

- Detect large molecules with low volatility
- No sample handling to reduce confounding variables

METHODS: A SUPER SESI and an Exhalion Capnography unit were coupled to an Orbitrap QE Plus mass spectrometer. Temperatures were set at 130º C (sample line) and 95º C (ionization region). The nano-electrospray was stabilized at 60 nA, and the flow passing through the SUPER SESI was set at 250 ml/min. Subjects exhaled through a medical grade spirometry filter to eliminate aerosols and patogens. CO_2 exhaled flow rate and exhaled volume data was aquired by Exhalion. Larger metabolites were ionized by SUPER SESI, and their signals were recorded by the MS with a time resolution of 0.2 seconds. A total of 100 exhalations from 3 people were analyzed for 5 months.

Online real-time analysis of breath with SUPER SESI-HRMS

- SUPER SESI is an ion source optimized for High Resolution Mass Spectrometry (HRMS) analysis of low volatility metabolites in the gas phase and in real time.
- EXHALION is a capnography system that measures CO_2, exhaled flow and volume.

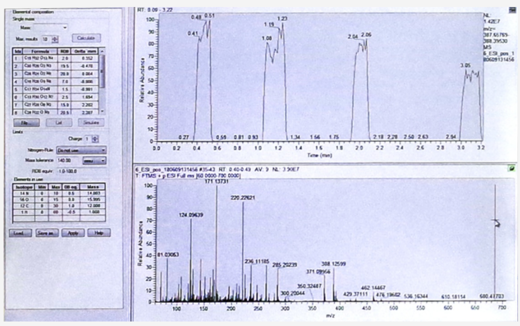
Follow these simple steps to turn your MS into a powerful advanced capnography system:

1 Couple SUPER SESI and Exhalion to your mass spectrometer (MS).

2 Set parametres and wait for the system to stabilize.

3 Exhale and check that signals are steady and breath is properly detected.

4 Start aquiring data. It is stored in the MS computer and Exhalion (USB).



RESULTS: Data was post-processed with the dedicated software (SW) Ariadne. This SW first detects edges in the Total Ion Count (TIC) signal to identify exhalations. Figure 1 shows the time evolution of the TIC, which goes up when the subject exhales, and returns to background levels when the exhalation is finished. The signal for 4-hydroxy-2-hexadecenal is also shown here to illustrate an example of detected low volatility species (its vapor pressure at 36ºC is $2.7 \cdot 10^{-7}$ Bar).

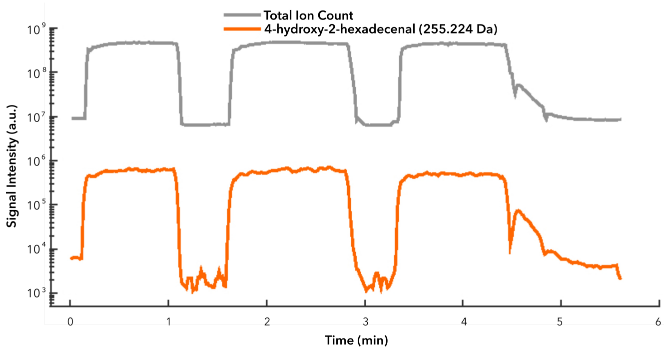


Figure 1. Time evolution of the signals detected by the SUPER SESI-MS

After this, Ariadne calculates the time averaged spectra measured in the background (before each exhalation), and during the exhalations, and builds a list of peak centroids with their respective signal intensity.

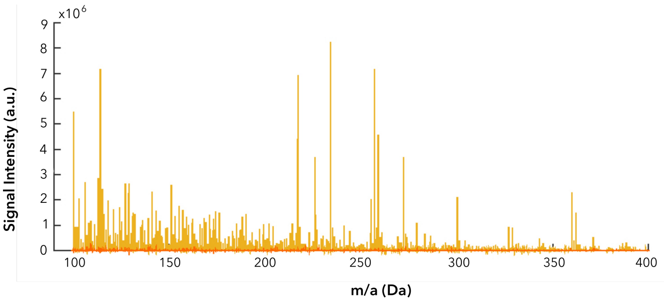


Figure 2. Averaged spectra of exhaled breath (yellow) and background (orange).

In order to determine the number of peaks detected, Ariadne reorders the signal intensities of the centroids of each experiment from high to low. The signal intensity threshold is set at 1000 a.u. because signals below display an erratic behavior. The average number of peaks detected above this threshold was 8000 in the exhalation and 2000 in the background (Figure 3).

When statistically analyzing how the different peaks detected are distributed along the different mass ranges (see fig. 4), we found that most species detected in breath were in the range between 150 and 300 Da. Above 300 Da, the number of species detected started to decline. This is because larger molecules have lower volatilities, and fall below the limit of detection of the instrument. For masses above 400 Da, the number of species detected in breath and in the background overlap. This is because low volatility species tend to condensate in the inner walls of the system and produce memory effects, thus contributing to the background level, as illustrated in Fig.1 . In this figure, the background level for 4-hydroxy-2-hexadecenal is above the threshold. Nevertheless, most of these signals are still clearly differentiable.

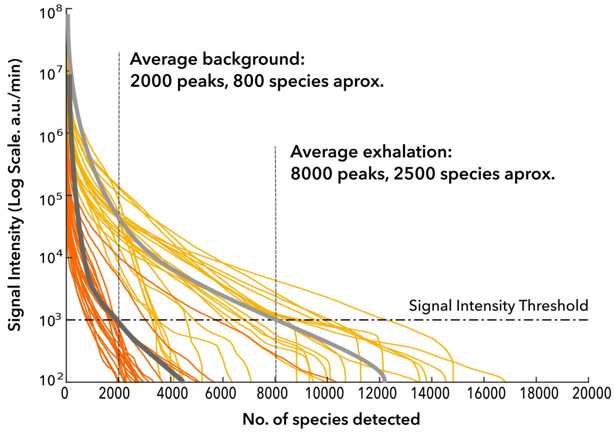


Figure 3. Each line represents signal intensities vs peak number ordered high to low in each experiment: highest signal is plotted as x=1, y= s1, second highest signal is x=2, y=s2, and so on. Yellow and orange lines correspond to breath and background respectively. The average results of all experiments is highlighted in different tones of grey.

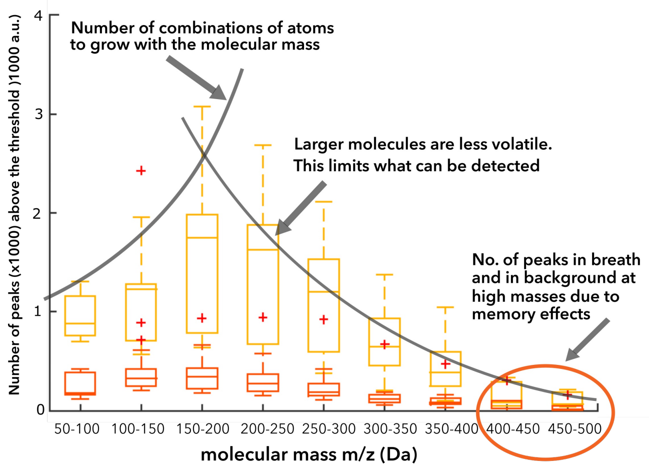


Figure 4. Represents the number of peaks detected above the threshold for the different molecular mass ranges.

CONCLUSIONS: Our initial hypothesis was that, if an instrument is sensitive down to the ppt range, it should be able to detect species with vapor pressures approaching 10^{-12} Bar. However, the general consensus is that very low volatility species are 'non-volatile' and thus 'non-detectable'.

- This application note shows that molecules with vapor pressures as low as $2.7 \cdot 10^{-7}$ Bar can be readily detected in breath in real time with SUPER SESI-HRMS.
- There is still a lot of room for improvement, but we are effectivelly expanding the mass range that can be detected.
- SUPER SESI - HRMS can detect thousands of species in breath, providing the exhalation profile in real time. This, combined with capnography data, opens a new non-invasive window to the analysis of human metabolism.

SUPER SESI

Why online real-time analysis of breath?

Every time we breath, we exhale thousands of molecular species that reflect our metabolism in that moment.

As evidenced by several efforts aiming at standarizing sample collection procedures, breath samples are very vulnerable to handling and sample treatment. This results into the introduction of undetected confounding variables in the data-sets, which hinder the identification of reliable and repeatable biomarkers.

Secondary Electro-Spray Ionization Mass Spectrometry (SESI-MS) enables the direct (online) analysis of exhaled breath, reducing the number of confounding variables and hence increasing the reliability of the obtained results. This shows the potential of breath analysis for the future of non-invasive early diagnosis.

Biological relevance

Larger molecules can be more specific to unique metabolic pathways. This makes them potentially more relevant from the biological point of view. However, molecules with higher molecular masses tend to have lower volatilities. For this reason, detecting them can be a challenge.

How does it work?

SUPER SESI is an ion source that operates at atmospheric pressure. It is coupled to the Mass Spectrometer (MS) exactly as the ion source of a MS. Patients and control groups direcly exhale into the SUPER SESI through a regular disposable spirometry antibacterial filter and breath is ionized in SUPER SESI. At high temperature, a nano Electro-Spray (nESI) produces a cloud of charging ions that ionize the vapor molecules of exhaled breath and transferred them to the Mass Spectrometer (MS).

SUPER SESI works together with EXHALION, a capnography system that collects the exhaled flow and displays real-time measurements of CO_2 concentrations and exhaled flow. It also calculates the exhaled volume, enabling to combine molecular data with capnography data, which provides valuable and validated information on the lung function. This shows the potential of breath analysis for the future of early diagnosis.

We believe that real-time detection and monitoring of complex metabolites in breath will enable countless new applications. We want to be your engineering partner.

What would you use it for?
Let us know at info@fossiliontech.com

Use cases

1. Identification of COPD patients by real-time breath anaylisis

Results: SESI-HRMS helped identifying biomarkers to determine if a patient suffers from COPD.

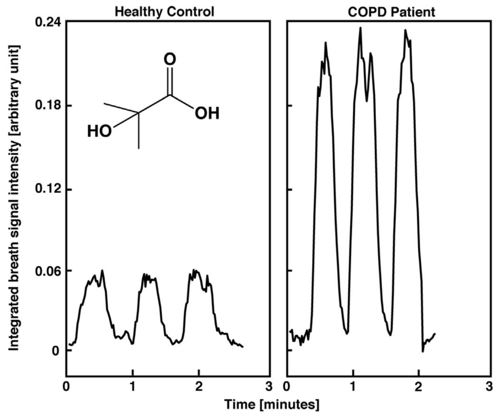


Figure 5. Comparison of average exhaled breath and background intensity of a COPD patient and a healthy control analysed with SESI-MS. A total of 43 biomarkers were identified from 1441 different features (22 COPD patients and 14 healthy controls). These markers were determined to be metabolites of oxidative stress processes resulting from lung muscle degradation.

2. Tryptophan metabolites detected in breath

Results: Detection in exhaled human breath of 20 low volatility metabolites of the Tryptophan pathway.

Name[a]	Formula	$[M + H]^+$
Anthranilate	$C_7H_7NO_2$	138.0550
Tryptamine	$C_{10}H_{12}N_2$	161.1073
4,8-Dihydroxyquinoline 4,6-Dihydroxyquinoline	$C_9H_7NO_2$	162.0550
3-Methyldioxyindole	$C_9H_9NO_2$	164.0706
Indole-3-acetate 5-Hydroxyindoleacetaldehyde	$C_{10}H_9NO_2$	176.0706
3-Hydroxykynurenamine 5-Hydroxykynurenamine	$C_9H_{12}N_2O_2$	181.0972
5-Methoxyindoleacetate	$C_{11}H_{11}NO_3$	206.0812
4-(2-Aminophenyl)-2,4-dioxobutanoate	$C_{10}H_9NO_4$	208.0604
L-Kynurenine	$C_{10}H_{12}N_2O_3$	209.0921
N-Acetylserotonin	$C_{12}H_{14}N_2O_2$	219.1128
5-Hydroxy-L-tryptophan	$C_{11}H_{12}N_2O_3$	221.0921
4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoate	$C_{10}H_9NO_5$	224.0554
3-Hydroxy-L-kynurenine 5-Hydroxykynurenine	$C_{10}H_{12}N_2O_4$	225.0870
N-Formylkynurenine	$C_{11}H_{12}N_2O_4$	237.0870
6-Hydroxymelatonin	$C_{13}H_{16}N_2O_3$	249.1234
Formyl-*N*-acetyl-5-methoxykynurenamine	$C_{13}H_{16}N_2O_4$	265.1183

[a] Compounds in boldface: identity confirmed by tandem HRMS or UHPLC.

Figure 6. List of metabolites of the tryptophan pathway detected in breath by SESI-HRMS. Indole-3-acetate and 3-hydroxykynurenine were confirmed by comparing UHPLC-HRMS from standards and EBC. It is interesting the detection of N-Acetylserotonin and 6-hydroxymelatonin, that are two metabolites in the melatonin branch, known to have a role as antioxidant and neuroprotector, and to be involved in the entrainment of the circadian rhythms.

REFERENCES

(1) **REAL-TIME MASS SPECTROMETRIC IDENTIFICATION OF METABOLITES CHARACTERISTIC OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN EXHALED BREATH.** *L. Bregya, Y Nussbaumer-Ochsnerb, P. M-L Sinues, D. García-Gómez, Y. Suter, T. Gaisl, N. Stebler, M. T. Gaugg, M. Kohler, R. Zenobi*

(2) **SESI-HRMS REVEALS TRYPTOPHAN PATHWAY METABOLITES IN EXHALED HUMAN BREATH.** D. García-Gómez, T. Gaisl, L. Bregy, P. M-L Sinues, M. Kohler and R. Zenobi