SUPER **SESI**

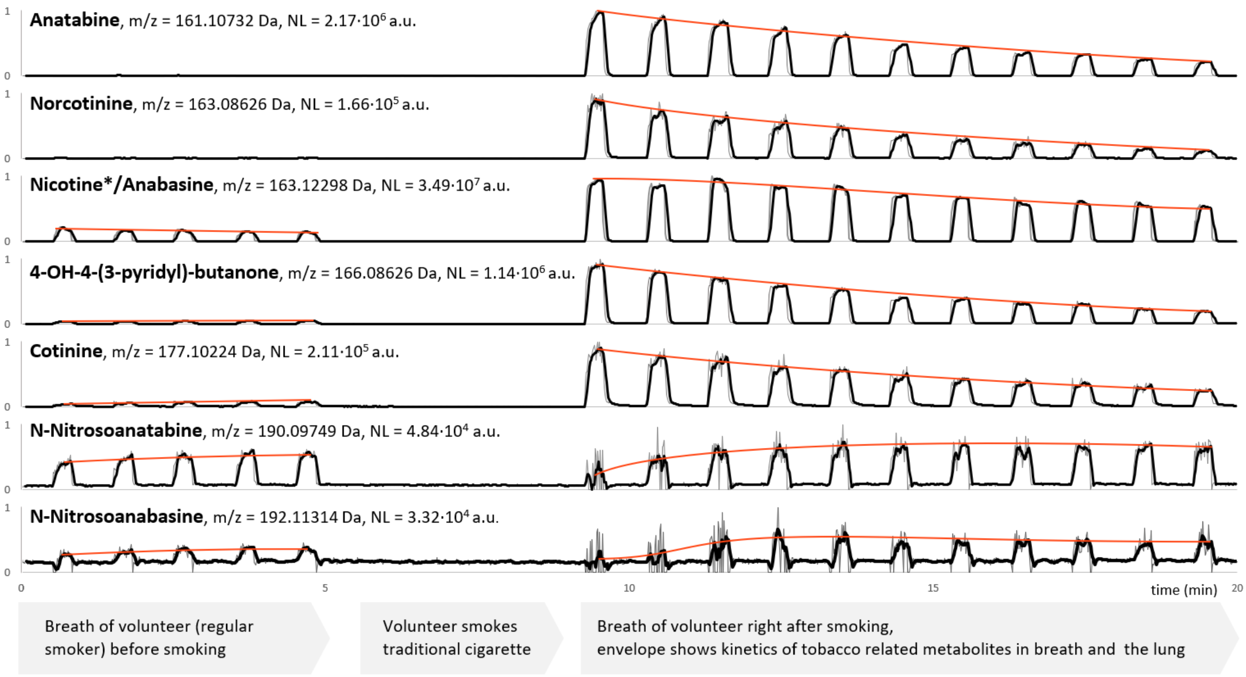
(Application note)

FOSSILIONTECH

Monitoring kinetics of tobacco metabolites by breath analysis

An alternative way to study metabolite delivery from the lung into the bloodstream.

Methods: Breath was analyzed for 20 min with a SUPER SESI - QExactive HF - EXHALION system. At min 5, the volunteer left the lab to smoke a cigarette. Min 10 to 20 show the time evolution of different tobacco related metabolites. Conditions: one exhalation per minute, exhaled volume 2 L, flow 7 lpm, high resolution, full scan 160-200 Da. The antibacterial filter was removed for a better time response. A set of tobacco related metabolites that were previously identified in smoker's urine by LC-MS-MS where monitored in real time.



* In a subsequent test, the mass 163 was fragmented. The 163.12298 signal corresponded to Nicotine.

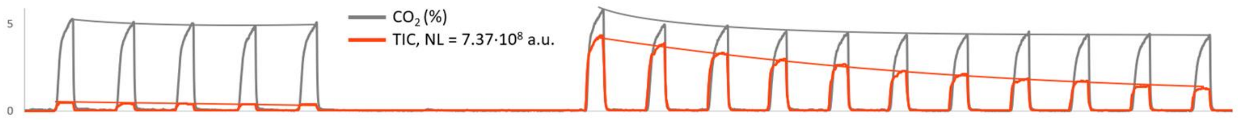
Results: Of the metabolites of interest, two distinct trends were observed: **anatabine**, **nornicotine**, **nicotine**, **4-OH-4-(3-Pyridyl)-butanone** and **cotinine**, which are detectable in tobacco [1], showed a washing pattern, where intensity rises right after smoking, and slowly decreases afterwards, each compound showing a different lifetime in the lung. In contrast, **N-nitrosoanatabine** and **N-nitrosoanabasine**, which are products of anatabine and anabasine metabolism [2], showed a comparably higher baselevel before smoking, a significant drop right after smoking, and an increase in the following exhalations.

SUPER **SESI**

(Application note)

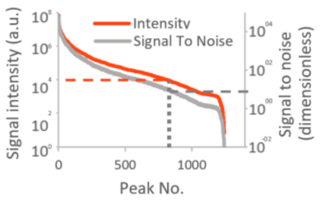
For the observed metabolites, washing in the lung is dominated by uptake into the bloodstream because they cannot evaporate so quickly.

The Total Ion Count (TIC) signal shows a well-defined washing pattern. This shows that many molecules can be traced, and their uptake kinetics characterized.



These curves provide an alternative way to estimate the velocity at which molecules are delivered from the lung into the bloodstream after smoking. Interestingly, previous nicotine absorption rate measurements [3] by blood analysis and our results by breath analysis show a comparable time-scale.

The number of peaks detected was consistent with previous studies [4]. With a Signal to Noise (S/N) of 30, about 800 peaks appear in the analyzed mass range with a dynamic range of 10^4.

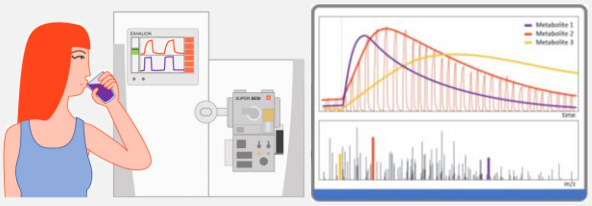


Conclusions:

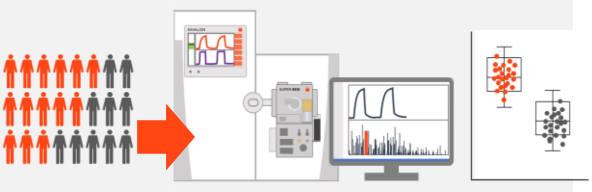
These experiments show that many tobacco related metabolites normally found in urine can be also detected in breath. Urine and breath analysis are complementary tests. For instance, N-nitrosonornicotine was detectable in urine but not in breath. While urine provides information on the downstream metabolism of tobacco, real time breath analysis provides useful information on the exposure of the lung, and the absorption of molecules delivered through the lung into the bloodstream. Here we focused on 7 known molecules, but our data shows up to 800 peaks that were washing after smoking. This is to be expected, as smoke is a highly complex cocktail.

This application note illustrates how breath is a very dynamic process. It also shows how real-time breath analysis can be used to study the kinetics of lung uptake and the metabolic response of the body in a non-invasive way. Other applications of real time breath analysis include biomarker identification, kinetics of drug metabolism, and kinetics of lung delivered drug uptake.

Non-invasive kinetic monitoring



Breath Biomarker Identification



References:

[1] Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke; S. S. Hecht and D. Hoffmann; Carcinogenesis vol.9 no.6 pp.875-884, 1988

[2] Metabolism and Disposition Kinetics of Nicotine; J. Hukkanen, P. Jacob III & N. L. Benowitz; *Pharmacol Rev* 57:79–115, 2005

[3] Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum; N. L. Benowitz, H. Porchet, L. Sheiner, & P. Jacob III,; Clinical Pharmacology & Therapeutics · August 1988

[4] SUPER SESI Application Note 2, No. of Peaks detected, Fossil Ion Technology