SUPER **SESI**

(Application note)

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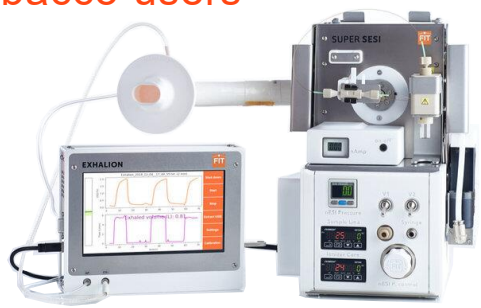
Monitoring of metabolite kinetics of tobacco users by real-time exhaled breath analysis

An alternative way to study delivery from the lungs into the bloodstream



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Objective: We hypothesized that real-time breath analysis can be used to monitor the kinetics of metabolites originating from tobacco and to recognize different time trends. To test this hypothesis, we focused on the following well-characterized molecules that are known to be present in the breath of smokers: **Nicotine**, a primary metabolite of interest in tobacco [1]; **4-hydroxy-2-hexenal**, known to be present in the breath of smokers and non-smokers and used for standardizing Secondary Electrospray Ionization coupled to High Resolution Mass Spectrometry (SESI–HRMS) breath testing procedures [2]; and **Indole**, an endogenous metabolite widely studied in breath [3].

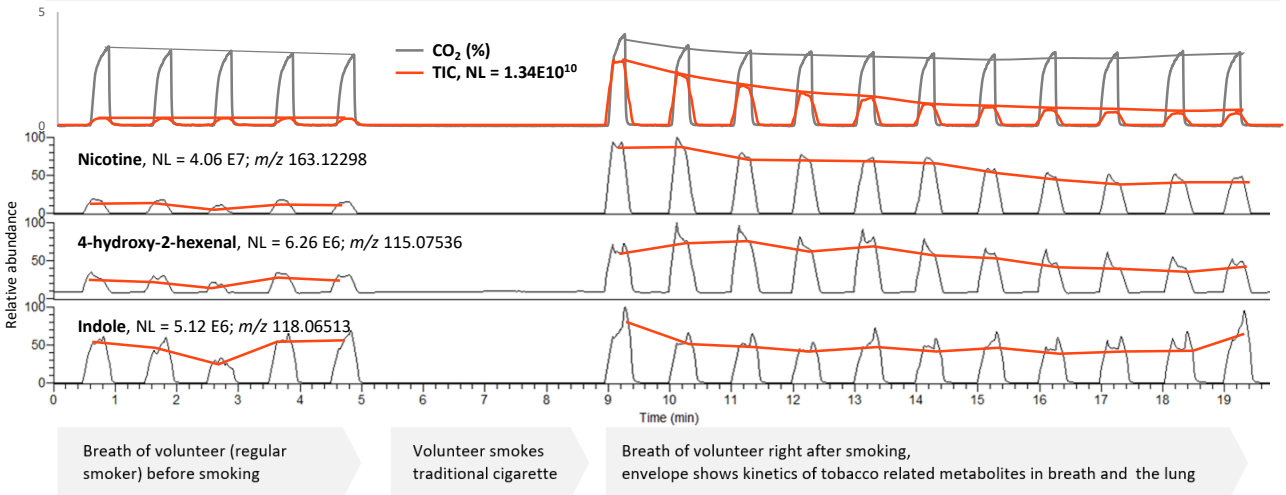
Protocol: Exhaled breath was analyzed for 20 min by using a SUPER SESI - QExactive HF - EXHALION system.

From 0 to 5 min: A volunteer exhaled 5 replicates (smoking abstinence for 30 min before the test).

At 5 min: The volunteer left the lab to smoke a cigarette.

From 9 to 20 min: The volunteer exhaled 11 replicates.

Conditions: One exhalation per minute (exhaled volume: 2 L; flow: 7 L/min). Exhaled CO_2 level was monitored. MS acquisition range *m/z: 50–600*; resolving power: 240,000; mass tolerance, 5 ppm.



Results, time trends: The total ion count (TIC) showed a well-defined washing pattern, compounded by several hundred peaks, which are so far unidentified. This was expected because tobacco smoke is a very complex mixture.

Distinct trends were observed in many of the metabolites detected. In particular,

- Nicotine showed a washing pattern, increasing right after smoking and slowly decreasing afterwards.
- 4-hydroxy-2-hexenal, which is known to be in the breath of smokers and non-smokers alike [2], also increased after smoking; but, its relative change was lower, and the washing pattern was less evident.
- Indole showed a relatively flat profile, where its levels did not seem to be affected by the smoking event.

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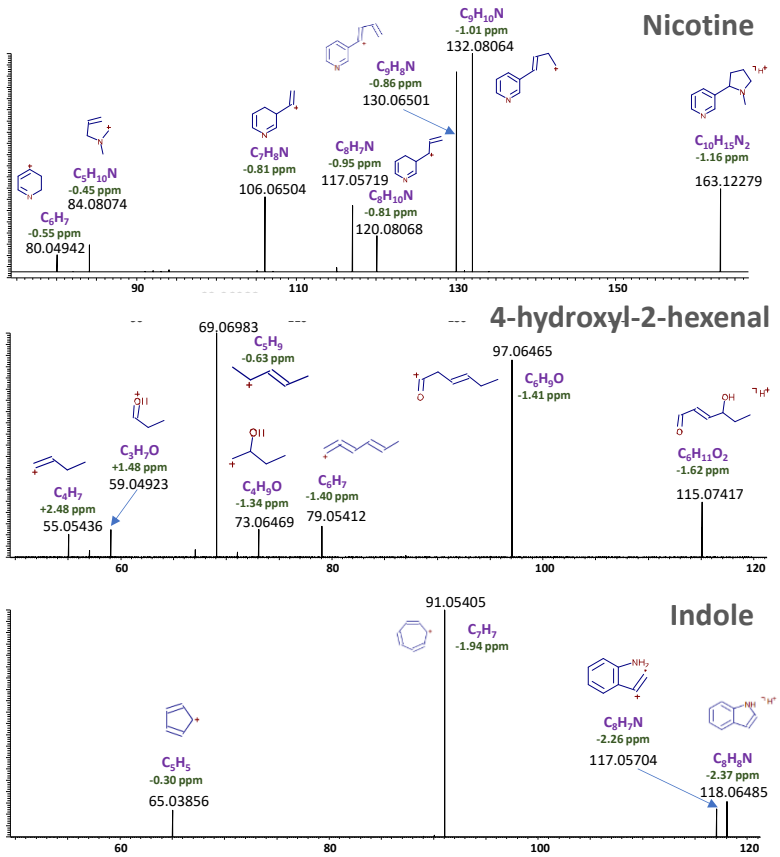
Results, identification: Putative identification of nicotine, 4-hydroxyl-2-hexenal, and indole was supported by the mass accuracy findings of the instrument (5 ppm window) and further confirmed by tandem MS/HRMS spectra by using high-energy collisional dissociation (HCD) to induce fragmentation of selected ions. All fragment ions were detected with high accuracy (below 1.2 ppm for nicotine fragments and 2.5 ppm for 4-hydroxyl-2-hexenal and indole fragments). Furthermore, these molecules have been previously reported to be present in breath and identified by LC–MS/HRMS of breath condensate.



Discussion: The fact that the time evolution of the three identified molecules is coherent with their origin (washing pattern for nicotine, a flat trend for the endogenous metabolite indole, and an intermediate trend for 4-hydroxyl-2-hexenal) indicates that real-time breath analysis can be used to capture the kinetics of these metabolites in the lungs.

Because the concentration of nicotine in breath is well below the ppm level [4], washing in the lungs is dominated by uptake into the bloodstream. These profiles provide an alternative way for estimating the velocity at which molecules are delivered from the lungs into the bloodstream after smoking. Interestingly, previous nicotine absorption rate measurements captured via blood analysis [1] show a comparable time scale with our breath analysis.

Conclusions:

These experiments show that many molecules can be traced and their uptake kinetics characterized. With regard to absorption of molecules delivered through the lungs into the bloodstream, real-time breath analysis provides useful information before and after exposure of the lungs. Our results show that, except compounds already known to be related with smoking, several peaks could contribute to the possible discovery of new biomarkers of smoking. This application note illustrates how breathing 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.

References:

[1] 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*, **1988**, *44*, 1, 23-28.

[2] Identification of 2-Alkenals, 4-Hydroxy-2-alkenals, and 4-Hydroxy-2,6-alkadienals in Exhaled Breath Condensate by UHPLC-HRMS and in Breath by Real-Time HRMS; D. García-Gómez, P. Sinues, C. Barrios, G. Vidal-de Miguel, M. Gaugg, and R. Zenobi; *Anal. Chem.* **2015**, *87*, 5, 3087-3093.

[3] Expanding metabolite coverage of real-time breath analysis by coupling a universal secondary electrospray ionization source and high resolution mass spectrometry—a pilot study on tobacco smokers; M. T. Gaugg, D. Garcia Gomez, C. Barrios-Collado, G. Vidal-de-Miguel, M. Kohler, R. Zenobi & P. Martinez-Lozano Sinues; *J. Breath Res.* **2016**, *10*, 1, 016010. doi:10.1088/1752-7155/101/016010.

[4] An Experimental Method to Determine the Concentration of Nicotine in Exhaled Breath and its Retention Rate Following Use of an Electronic Cigarette; G. O'Connell, S. Colard, K. Breiev, P. Sulzer, S. Biel, X. Cahours, J. D Pritchard and K. MM Burseg; *J. Environ. Anal. Chem.* **2015**, *2*, 5, doi: 10.4172/2380-2391.2391.1000161.