# Development of a Real-time Breath Analysis Platform and Applications: Diagnosis in Humans and Drug Monitoring in Mice


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## 1. Overview

- Exhaled breath contains relevant metabolites that may reflect the biochemical activity within a subject.
- However, in contrast to other biofluids (e.g. plasma), the analysis of breath remains far less explored.
- Secondary Electrospray Ionization (SESI) in tandem with Atmospheric Pressure ionization Mass Spectrometry (API MS) has achieved sensitivities in the sub-ppt range for polar vapors such as drugs, explosives and breath [1-7].
- Low-Flow SESI (LF-SESI), was developed in tandem with a Differential Mobility Analyzer (DMA), and in explosive detection applications reached sensitivities at the sub-ppq range [8-9]. Figure 1 shows the LFSESI configuration.
- We developed a real-time breath analysis platform via MS by implementing an add-on LFSESI [10] on a commercial mass spectrometer atmospheric pressure inlet. Here we present some applications.

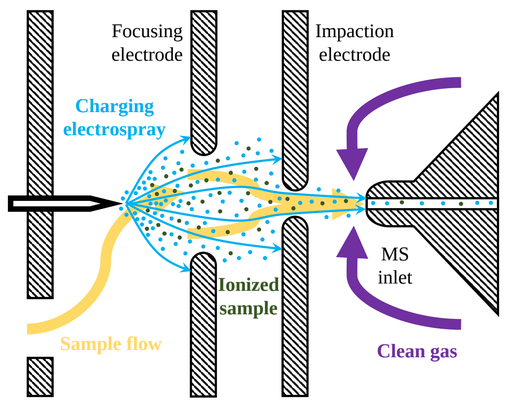
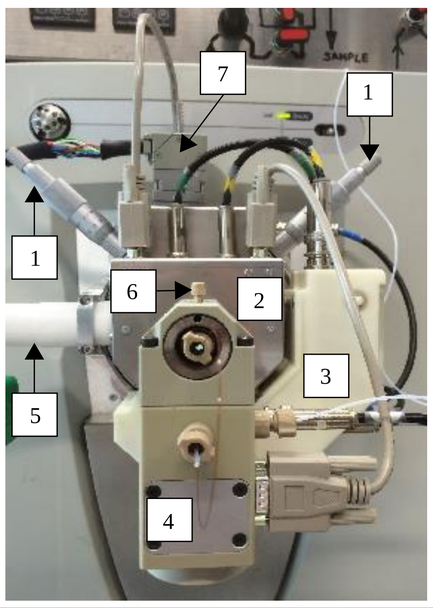



*Figure 1 LFSESI configuration: Electrospray ions (blue) transfer charge to sample vapors (yellow) which become ionized (green) and get into the MS.*

## 2. Breath analyzer architecture

- The breath analysis platform consists basically on a heated asymmetric LFSESI chamber which is coupled to the MS atmospheric pressure interface. A 2-axis micrometric positioning system provides fine mechanical alignment between the LFSESI and the MS inlet capillary. Also, the axial position of the electrospray tip can be optimized manually. Figure 2 shows the LFSESI coupled to an MS.



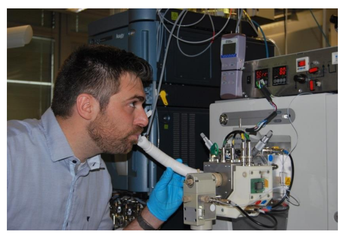



*Figure 2 Orbitrap Mass Spectrometer with LFSESI [10]: 1. Mechanical alignment; 2. Thermal insulation surrounding the core; 3. High voltage electronics; 4. Electrospray vial holder; 5. Transfer line; 6. Electrospray positioning; 7. Temperature control connection for core and transfer line*

## 3. Pilot tests: smokers

- First real breath analysis with the new platform was a pilot test with smokers and non-smokers [11].
- Around 1000 features were detected.
- Compounds over 900 Da were detected, which expands the available state-of-the-art on-line breath analyzer range.
- We found compounds correlated with smoking frequency.
- Breathprints allowed 100% accuracy at smoking/non-smoking status prediction.

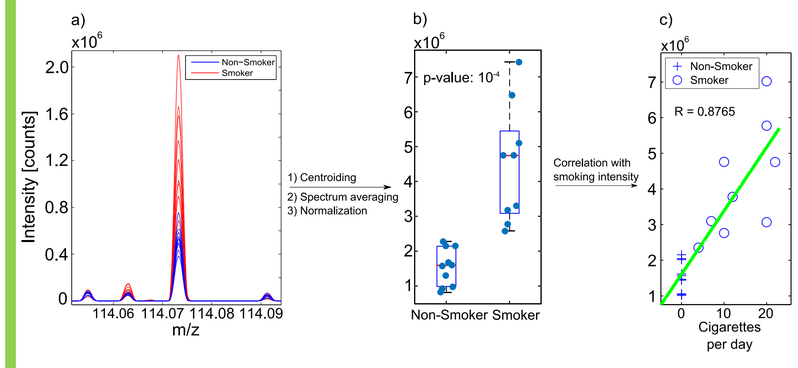



*Figure 3 Differences between smokers and non-smokers; a) overlaid breath mass spectra of all subjects in the region around the feature at m/z 114.0733; b) box-plot of the average intensities per subject, split into smokers and non-smokers. This compound was significantly increased in smokers; c) linear regression between the peak intensities of the feature at m/z 114.0733 and smoking frequency.*

## 4. Drugs detection

- Sensitivity was tested towards vapors of common drugs [10].
- Drugs were injected into a nitrogen flow of *0.2 L/min*.
- Figure 4 shows that the system was able to detect these drugs from concentrations of tenths of *ppt* in the gas phase, with a linear response across three orders of magnitude.
- Such low concentrations are deemed to be necessary to be detected in exhaled breath of small animals such as mice.

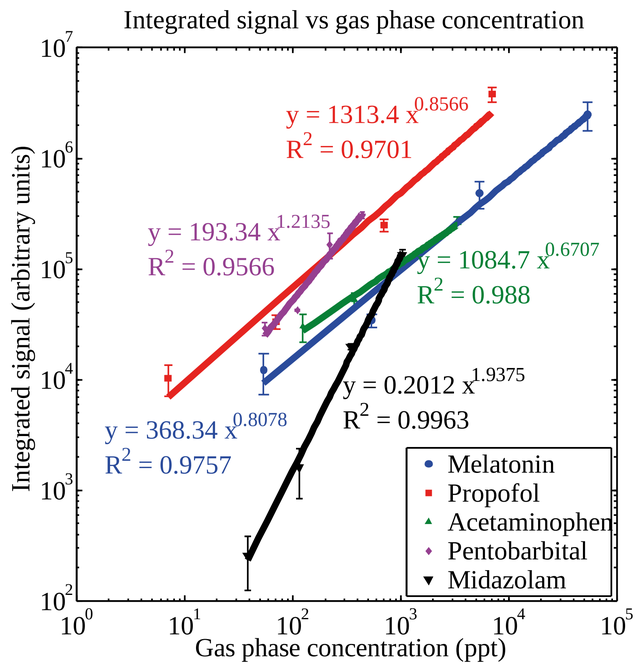



*Figure 4 Detection of several drugs of interest*

## 5. Analysis of aldehydes

- Aldehydes and furans in breath in real time were studied [12,13].
- High volatile aldehydes (less than six carbon atoms), not detected in exhaled breath condensate studies, were identified.
- Figure 5 shows detection of aldehydes in breath in real time.




*Figure 5 Real time analysis of aldehyde in Exhaled Breath Condensate [12]*

## 6. Diagnosis of OSA

- We have studied obstructive sleep apnea (OSA) in a randomized controlled trial [14].
- We found a panel of breath metabolites that were significantly enhanced in breath after treatment withdrawal. Figure 6 shows a particular example (pentenal). Further identification of the compounds enabled gaining insights into OSA.

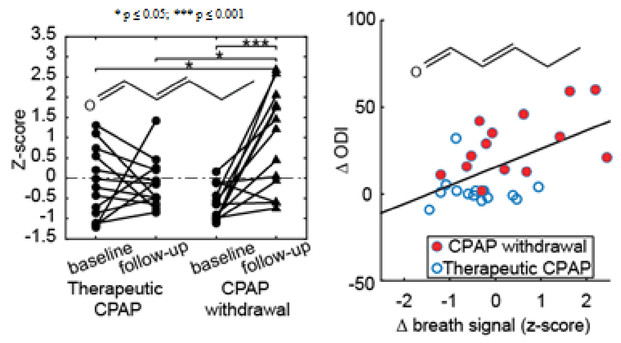



*Figure 6 Breath levels of pentenal in patients with OSA [14]*

## 7. Drug monitoring in mice breath

- We have studied breath levels of ketamine and other drugs in mice injected with these substances [15].
- Figure 7 shows time-dependent ketamine signal for four different doses: 15, 30, 45 and 60 mg/kg.
- Each dose was injected in different mice (n=4).

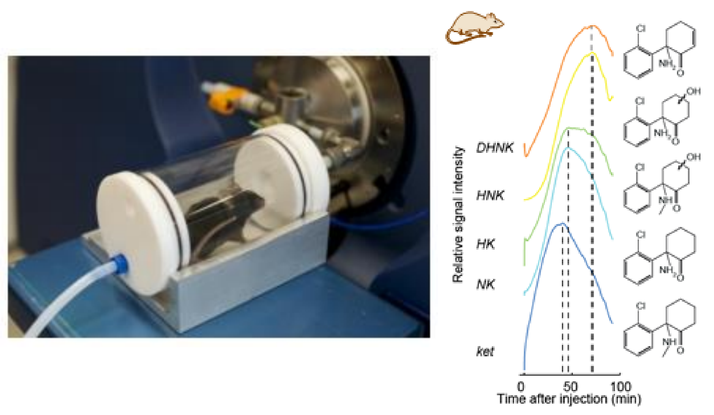



*Figure 7 Breath levels of ketamine in mice injected with ketamine [15]*

## 8. Conclusions

- We conclude that the real-time mass spectrometric analysis of exhaled metabolites may contribute to address some of the most relevant clinical and pharmacological problems, which are currently investigated through the analysis of body fluids other than breath.
- We developed a real-time breath analysis platform which allows *In vivo* monitoring of exhaled compounds.

## 9. Acknowledgements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## 10. References


1. C. Wu, W. F. Siems, H. H. Hill Jr. *Secondary Electrospray Ionization Ion Mobility Spectrometry / Mass Spectrometry of Illicit Drugs*. Anal. Chem 72, no. 2 (2000).
2. J. F de la Mora. *Ionization of Vapor Molecules by an Electrospray Cloud*. Int. J. Mass Spectrometry 300, no. 2-3 (2011)
3. P. Martínez-Lozano, J. Rus, G. F de la Mora, M. Hernández, J. F de la Mora*; Secondary Electrospray Ionization (SESI) of ambient vapors for explosive detection at concentrations bellow parts per trillion*. American J. Mass Spectrometry (2009).
4. L. Meier, C. Berchtold, S. Schmid and R. Zenobi; Sensitive detection of drug vapors using an ion funnel interface for secondary electrospray ionization mass spectrometry; J. of Mass Spectrometry, 2012 May;47(5):555-9
5. M. Tam, H. H. Hill Jr. *Secondary Electrospray Ionization – Ion Mobility Spectrometry for Explosive Vapor Detection*. Anal. Chem. 76, 2741-2742 (2004).
6. P. Martínez-Lozano, J. Fernández de la Mora. *Electrospray ionization of volatiles in breath*. Int. J. Mass Spectrometry, 265 (1):68-72 (2007).
7. C. Berchtold, L. Meier, R. Zenobi. *Evaluation of Extractive Electrospray Ionization and atmospheric pressure chemical ionization for the detection of narcotics in breath*. Int. J. Mass Spectrometry, vol. 299, issues 2-3, p. 145-150 (2011).
8. G. Vidal-de-Miguel, M. Macía, P. Pinacho, et. al. *Low Sample Flow Secondary Electro-Spray Ionization, improving vapor ionization efficiency*. Anal. Chem. (2012).
9. G. Vidal-de-Miguel, D. Zamora, M. Amo, A. Casado, G. F de la Mora, J. F de la Mora; *Method for Detecting Atmospheric Vapors at Parts per Quadrillion (PPQ) Concentrations.* USPTO
10. C. Barrios-Collado, G. Vidal-de-Miguel, P. Martínez-Lozano Sinues; *Numerical Modelling and Experimental Validation of a Universal Secondary Electrospray Ionization Source for Mass Spectrometric Gas Analysis in Real-Time*. Submitted to Sensors and Actuators B: Chemical.
11. M. Gaugg, D. García-Gómez, C. Barrios-Collado, G. Vidal-de-Miguel, M. Kohler, R. Zenobi, P. Martinez-Lozano Sinues; *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*. Submitted to J. Breath Research
12. D. García-Gómez, P. Martínez-Lozano, C. Barrios-Collado, G. Vidal-de-Miguel, M. Gaugg, R. Zenobi; *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.* Anal. Chem. (2015)
13. D. García-Gómez, L. Bregy, C. Barrios-Collado, G. Vidal-de-Miguel, R. Zenobi *Real-time high-resolution tandem mass spectrometry identifies furan derivatives in exhaled breath.* Anal. Chem. (2015)
14. E. I. Schwarz, P. Martinez-Lozano Sinues, L. Bregy, T. Gaisl, M. Gaugg, Y. Nussbaumer-Ochsner, J. R. Stradling, R. Zenobi, M. Kohler; *The Effect of CPAP Withdrawal on Exhaled Breath in Obstructive Sleep Apnea – A Randomized Controlled Trial*. Anual Meeting of the Swiss Respiratory Society, Lugano, Switzerland April 2015
15. X. Li, P. Martínez-Lozano Sinues, R. Dallmann, L. Bregy, M. Hollmén, S. Proulx, et al., *Drug pharmacokinetics Determined by Real-Time Analysis of Mouse Breath* Angew Crem Int Ed (2015)



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