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

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.
- Low-Flow SESI (LF-SESI) was developed in tandem with a Differential Mobility Analyzer (DMA), and in explosive detection, reached sensitivities at the sub-ppt range [3-8].
- We developed a real-time breath analysis platform via MS by implementing an add-on LF-SESI [10] on a commercial mass spectrometer atmospheric pressure inlet. Here we present some applications.

2. Breath analyzer architecture

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

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.

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 these orders of magnitude.
- Such low concentrations are deemed to be necessary to be detected in exhaled breath of small animals such as mice.

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.

6. Diagnosis of OSA

- We have studied obstructive sleep apnea (OSA) in a randomized controlled trial [4].
- We found a panel of breath metabolites that were significantly enhanced in breath after treatment withdrawal. Figure 6 shows a particular example (pentanal), further identification of the compounds enabled gaining insights into OSA.

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=6].

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.

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10. References