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Proton Transfer Reaction Time of Flight Mass Spectrometry (PTR-ToF-MS) for in vivo breath analysis of rats under different diet conditions

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Summary: The present study demonstrates the possibility to measure online the exhaled breath of awake rats. The potentiality of PTR-ToF-MS have been tested providing analytical information that allows the discrimination of subjects according the diet regime demonstrating the possibility to monitor the metabolic state of subjects through fast non invasive breath analysis.

Keywords: proton transfer reaction-mass spectrometry; breath analysis; diet.

1 Introduction

Volatile compound found in exhaled breath may provide information on the physiology state of subjects. Rats are commonly used in preclinical trials to investigate the effect of the diet on the animal health before test in humans. Analysis of the component present in the breath of such animals can give important information on state of their metabolism and may bring to the identification of markers specific for the different metabolic conditions.
Recently PTR-MS has been proposed for the rapid screening of potential pathologies markers [1]. Aim of the present study was to verify the feasibility of PTR-ToF-MS for the online *in vivo* analysis of rat breath to demonstrate the potential of such technique for animal breath analysis and to found possible markers associated at different diet regimes.

2 Experimental

Animals. Sixteen rats divided in 4 groups were used for the experiment. Each group was subjected to a different diet regime: "Nor.Wat" rats feed with standard pellets and water; "Nor.Cof" rats feed with standard pellets and decaffeinated coffee; "Fat.Wat" rats feed with fat supplement and water; "Fat.cof" rats feed with fat supplement and decaffeinated coffee.
Breath collection. Exhaled breath was sampled introducing the muzzle rat in a 50 ml Falcon™ conical tube that was directly connected to the PTR-MS inlet (Fig. 1). Breath collection was achieved for at least 20 seconds. Breath of all the animals was collected on the same day and the experiment was replicated after a week.
Food volatile compounds. Headspace of the coffee and of standard and fat supplement was determinated as well to verify the presence and the levels of the detected compounds and their possible interference with the concentration found in exhaled breath.

Fig. 1. Sampling of rat exhaled breath.

3 Results

Figure 2 reports a Principal Component Analysis (PCA) of all the collected data over the two experiment days. The four groups are well separated indicating that the exhaled breath composition is related to the different diet regimes. Both fat supplementation and coffee influenced the metabolic state of rats.

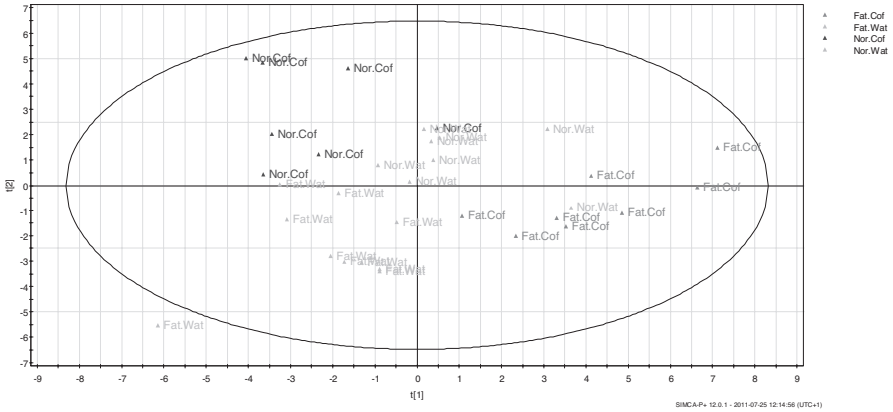

Fig. 2. PCA score plot of first 2 components of the all collected data (two days replicates are included).

Different masses were identified as potential markers. For example m/z 42.0336 corresponding to the protonated acetonitrile (theoretical mass 42.03382 Th) is high in the breath of rats fed with standard diet and even higher in rats drinking coffee (Fig. 3).

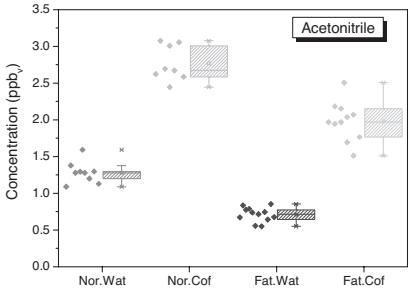

Fig. 3. Acetonitrile concentration in the rats' exhaled breath. Boxes: 25-75 percentile; whiskers: outliers; small boxes: mean value.

Concentration of methanol (m/z 33.0335) is higher in the breath of rats under standard diet regime and is not influenced by the coffee (Fig. 4).

Acetonitrile and methanol were both present at different concentrations in the headspace of coffee and of solids food. From the comparison with these data (not shown), the measurement of such compounds in the rats' breath seems not be influenced by supplemented foods.

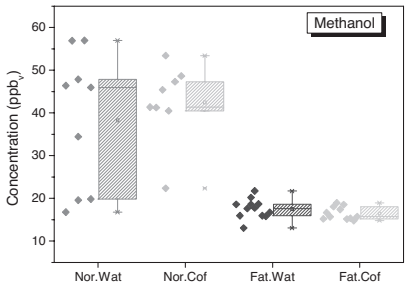

Fig. 4. Methanol concentration in the rats' exhaled breath.

4 Conclusions

This study demonstrated the possibility to analyze online the composition of exhaled breath of awake rats minimally affecting their metabolic state. Markers of diet regime can be identified by PTR-ToF-MS showing the potentiality of this technique for the monitoring of metabolic conditions of the mammals through the fast non-invasive breath analysis.

References

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