SIMULTANEOUS MULTIPLE SPME FIBERS SAMPLING TO EXTRACT SAMPLE FULL POTENTIAL

Thibaut **Dejong**[1], Kinjal **Bhatt**[1], Paulina K. **Piotrowski**[2], Pierre-Hugues **Stefanuto**[1], Jean-Francois **Focant**[1]

1 - University of Liège, Molecular Systems, Organic & Biological Analytical Chemistry Group, 11 Allée du Six Août, 4000 Liège, Belgium
2 – National Institute of Standards and Technology (NIST), Chemical Sciences Division, Gaithersburg, Maryland, USA

Key Points

• By placing the HS vial with the fiber at -20°C, we divided by a factor of two the RSD compared to storage at room temperature.

• The Pegasus™ BT 4D LECO detects more than **11 times more compounds** and is 100 times more sensitive than the Pegasus™ 4D HRT.

• The Pegasus™ 4D HRT system offers **stronger mass accuracy** around 2ppm and enable a more robust compound identification.

• **When dealing with complex-biological matrices, the ability to combine low limit of detection on one instrument and high MS accuracy on a second one represents a large added value.**

Context

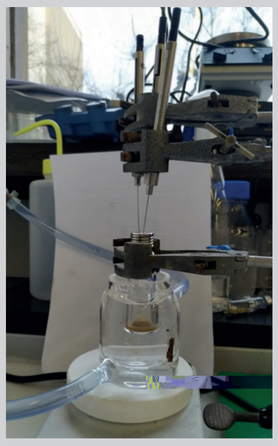
In omics research setting, access to sample is usually a key factor of the experimental design. In some cases, samples can be abundant and readily available. However, in the case of biological matrices, samples can be difficult to obtain and the chemical integrity difficult to maintain. In the context of microbiome research, stool samples are difficult to obtain, difficult to homogenize, difficult to store [1,2]. Unstable samples make difficult potential combinations of different analytical techniques without introducing bias. For example, when solid-phase micro extraction fiber (SPME) is employed for VOCs analysis, only one extraction is possible. If something goes wrong, the entire sample is lost.
Here we developped a multi-SPME set up allowing multiple analyses of a single sample.

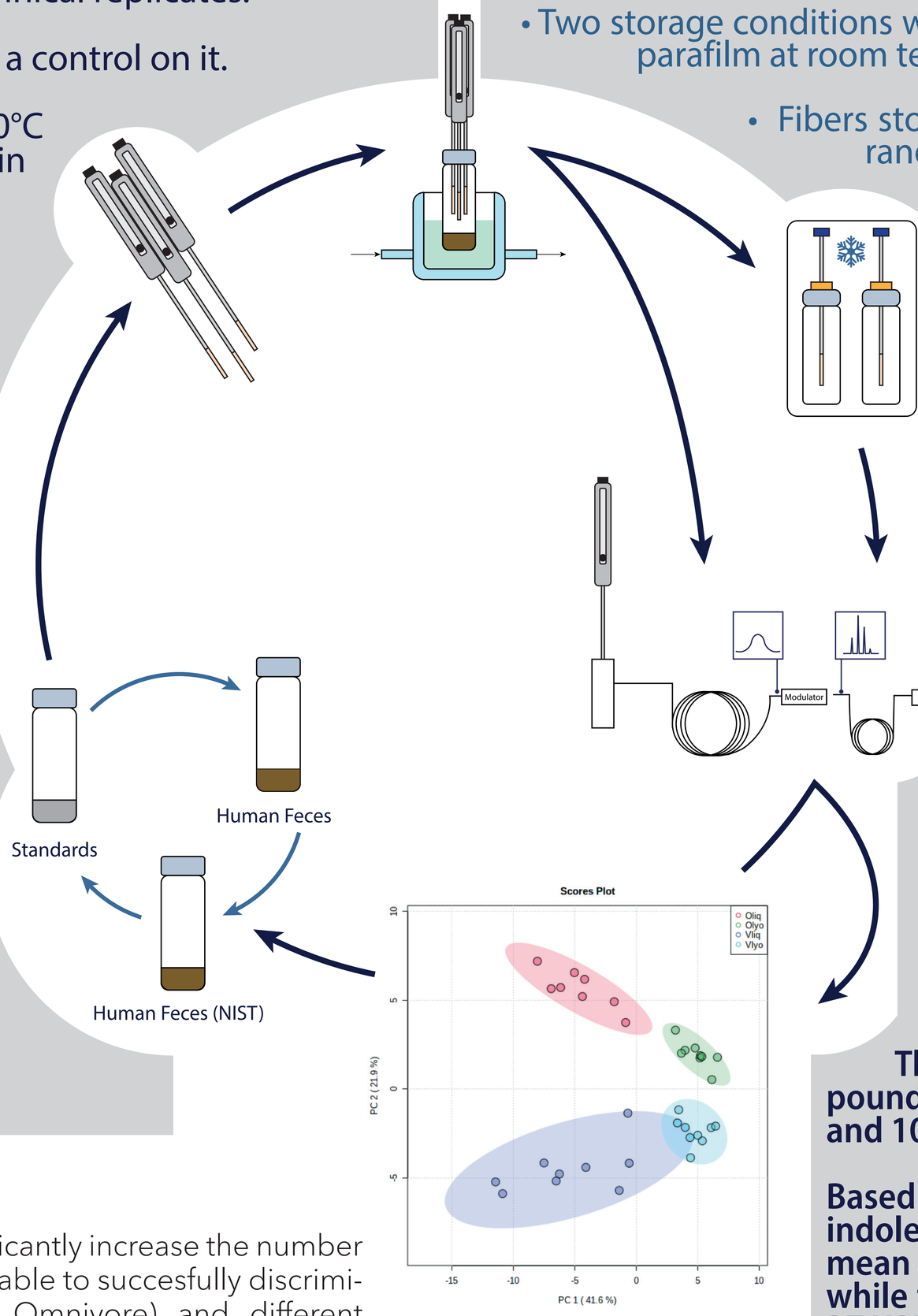
Method

• We used three Nitinol-core (NIT) Solid Phase Micro Extraction (SPME) fibers simultaneously on one single sample to generate three technical replicates.

• Fibers were labelled to keep a control on it.

• VOCs were extracted at 40°C for 20min after a 20min incubation.





Storage

• After the extraction, one fiber was analysed, the two others were stored.

• Two storage conditions were studied: in a HS vial with parafilm at room temperature and at -20°C.

• Fibers stored during the analysis were randomly selected.

The best storage condition was at -20°C with a RSD mean value of 19.8% compared to 38.4% for the storage condition at room temperature.

Samples

• We used three different kind of samples to test our method: a 24-standards mix, uncontrolled human feces samples and standard human feces samples provided by the NIST.

• 1mL of fecal sample was placed into Head Space (HS) vials.

Instrument

• Fibers were thermally desorbed and VOCs were analysed by GC×GC-(HR) TOFMS.

• Two complementary instruments were compared on the same biological sample, the Pegasus™ BT 4D and the Pegasus™ BT HRT.

The average number of compounds detected is 90 for the BT HRT and 1008 for the BT 4D.

Based on two fecal biomarkers, the indole and the benzaldehyde, the mean area for the BT 4D is around 10^7 while this value is around 10^5 for the BT HRT.

However, the BT HRT offers a stronger mass accuracy, for example, for the indole, 0.25 compared to 117.07 for the BT 4D.

Application Example:

This method was applied to significantly increase the number of sample from the NIST and was able to succesfully discriminate different diets (Vegan or Omnivore) and different sample preparation conditions (Liquid or Lyophilysed), as shown on the PCA where the PC1 and the PC2 explain 63.5 % of the total variance.

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[1] M.X. Chen, S.Y. Wang, C.H. Kuo, I.L. Tsai, Metabolome analysis for investigating host-gut microbiota interactions, J. Formos. Med. Assoc. 118 (2019) S10–S22. doi:10.1016/j.jfma.2018.09.007.

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Thibaut **Dejong**
tdejong@uliege.be