SIMULTANEOUS MULTIPLE SPME FIBER SAMPLING TO MAXIMIZE THE SAMPLE 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 into specific design glass tubes , we **divided by a factor of four** the RSD compared to storage at room temperature in HS vial.

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

• The Pegasus™ 4D HRT system offers **higher 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

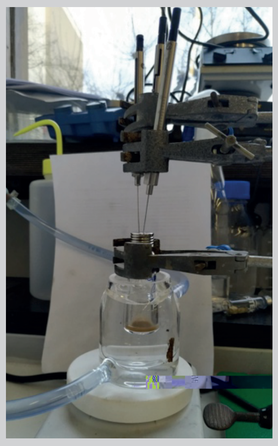
In omics research setting, access to sample is usually a key factor of the experimental design. In some cases, samples can be abundant and readily available. However, in the case of biological matrices, samples can be difficult to obtain and the chemical integrity difficult to maintain. In the context 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.

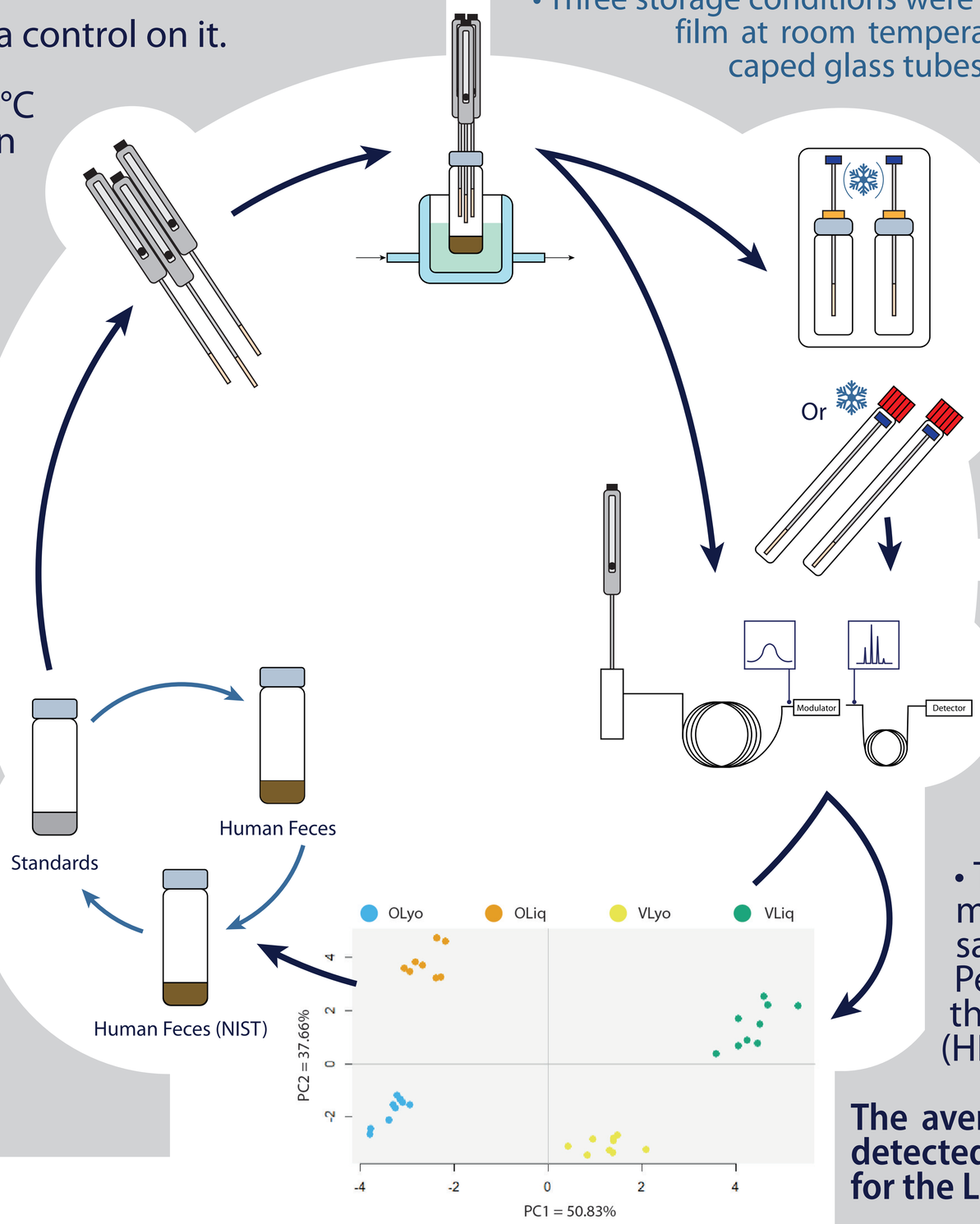
Method

• 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.



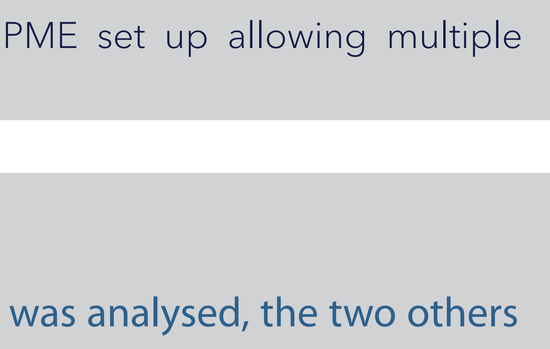


Storage

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

• Three storage conditions were studied: in a HS vial with para-film at room temperature, at -20°C and in specific caped glass tubes.

• Fibers were randomly selected.



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 (LRMS) and the Pegasus™ HRT 4D (HRMS).

The average number of compounds detected is 90 for the HRMS and 1008 for the LRMS.

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 88.49% of the total variance. This PCA was built on the top 20 compounds sorted by decreasing Fisher ratio.

**Based on two fecal biomarkers (indole and the benzaldehyde) the mean area for the LRMS is around 10^7 while this value is around 10^5 for the HRMS.
However, the HRMS offers a higher mass accuracy, for example, for the indole, 0.25 compared to 117.07 for the LRMS.**

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Thibaut **Dejong**
tdejong@uliege.be