Dried blood spot sampling: a new tool for NMR-based metabolomics approach

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Introduction:

The metabolomics study of whole blood has been since long time subject of several research because of the extremely diverse and complex nature of biological specimens. This field of "omics" sciences find a wide range of applications in personalized medicine approach going from clinical diagnostics for early disease detection, and from therapy prediction to the patient's follow-up. In the optic of the incoming personalized medicine era, the need for robust self-made bio-specimens' collection method is mandatory.

In this work a dried blood spot (DBS) device was tested for NMR-based metabolomics approach, with the main goal to enlarge the sampling available options for personalized methods approach. For this reasons our study can be summarize in the following key points:

- Set up of extraction method from DBS device
- Test repeatability and reproducibility of our extraction method
- Compare the DBS device with a classic plasma collection method
- Test our method in a "proof of concept study"

DBS device *versus* plasma collection: comparison study

In order to compare the developed protocol with the classical plasma collection method, a study based on a spiking experience was accomplished. A solution containing 10 metabolites three times more concentrated than in normal condition of blood stream, was prepared. After blood collection, to a first blood tube was added an aliquot of spiking solution before samples preparation , and a second one undergo directly to sample preparation and analysis ***(Figure 3)*** .

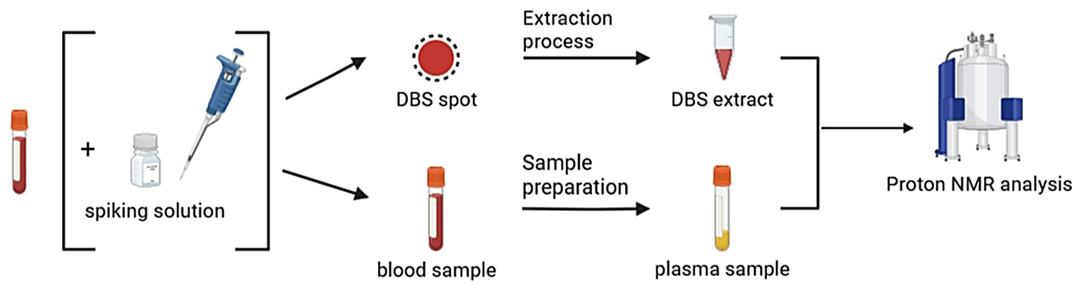

Figure 3: Pipeline for spiking experience

A multivariate statistical analysis was performed in way to compare the extraction from DBS device with the classic plasma method. The PLS-DA of both analysis ***(Figure4a-b)*** showed a good separation for the two models (Q^2=0.948 ; Q^2=0.946 for DBS and plasma respectively).The VIP scores for both models show the same list of relevant features for the separation ***(Figure4c-d)*** as proof of the fact that the extraction protocol works as well as the classical method.

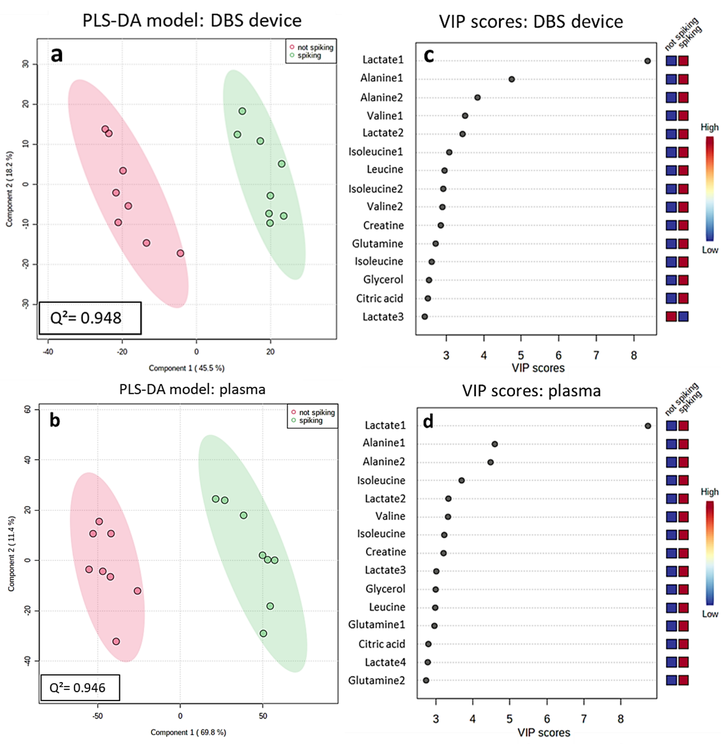

Figure 4 (a-b)PLS-DA models for DBS device and plasma (Q^2=0.948; Q^2=0.946 respectively); **(c-d)** related VIP scores

Metabolites identification and quantification was done through Chenomx NMR Suite in order to compare metabolomics profiles of a same individual coming from two different matrices. As we can see in the pie charts ***(Figure 5)*** the two sampling methods share the same main-classes of pathways with a 87% of plasma metabolites identified in DBS.

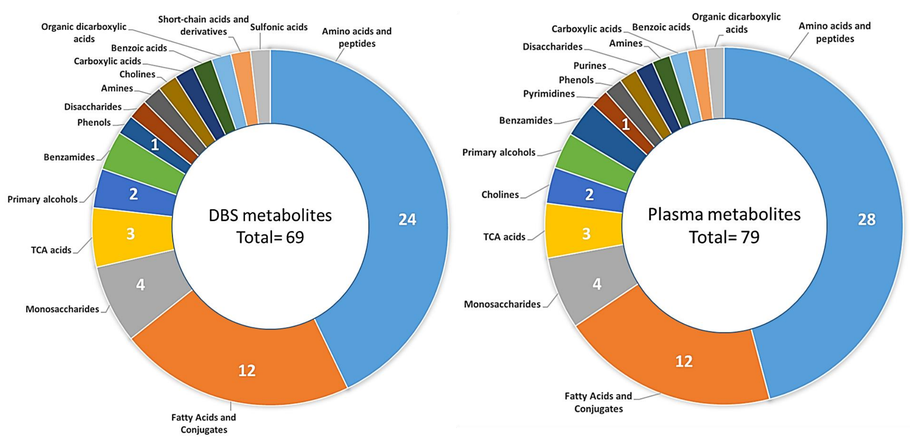

Figure 5: Main-classes of metabolites identified for DBS and plasma sampling

Methods:

1. Extraction method:

The extraction method was set up for obtain the most informative and quantitative ^{1}H-NMR spectra as following ***(Figure 1)*** :

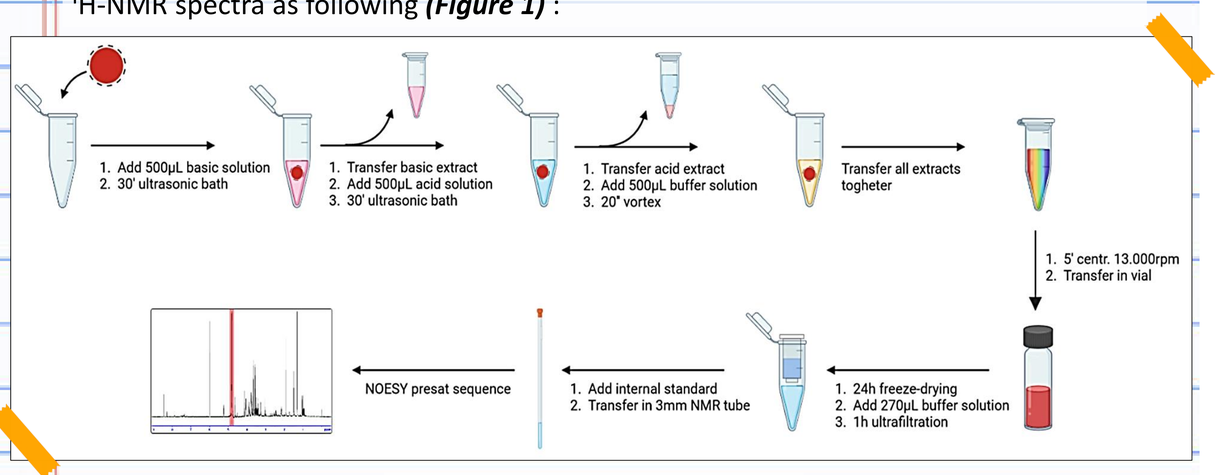

Figure 1: Extraction method's workflow

2. Repeatability and reproducibility :

In way to evaluate the metabolites' extraction repeatability of our method, 5 blood spots, coming from the same individual at a defined time point, were deposed on filter paper and extracted at the same time. Moreover, the reproducibility was estimate by deposing at the same time 15 blood spots then extracted at Day1, Day2 and Day3.

For reach this goal several metabolites were integrated and the coefficient of variation (CV%) was calculated. As we can see, in case of repeatability, all the metabolites are above the acceptance threshold of NMR technique (CV= 10%); different is the case of reproducibility in which we can see the acetate reach a value equal to 13.6 because of the bacterial contamination developed on the filter paper ***(Figure 2)*.**

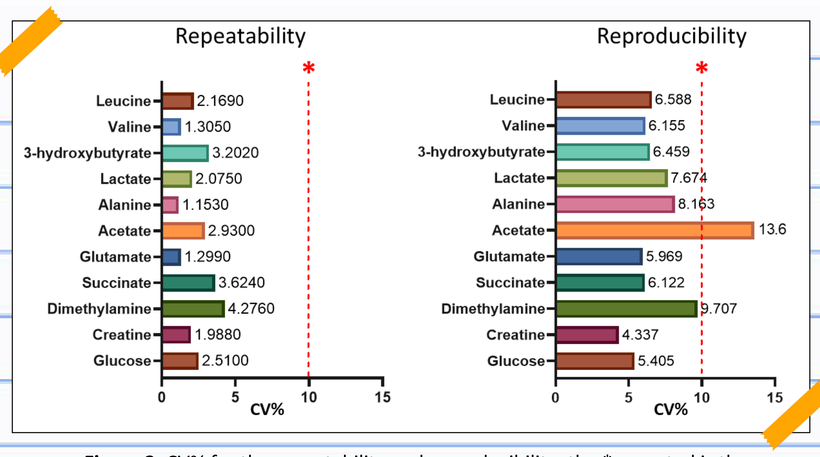

Figure 2: CV% for the repeatability and reproducibility; the * reported is the acceptance threshold relative to NMR technique

"Proof of concept" study

As proof of the concept, the method was applied to a real case study, by collecting DBS spots of female and male individuals for one week at two different time points: 30 minutes after eating and 1h after an intensive sport session ***(Figure 5***).

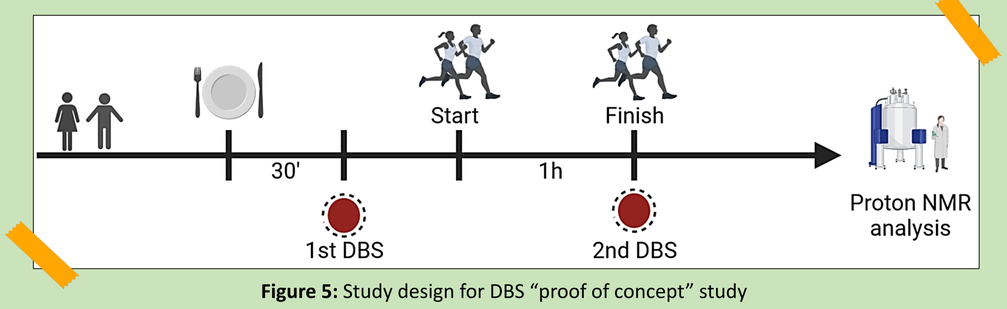

Figure 5: Study design for DBS "proof of concept" study

The multivariate discriminant analysis (PLS-DA, Q^2=0.937) for female individual, showed that a clear separation occurred between the two conditions ***(Figure 6a)***. As expected and illustrated by the VIP scores ***(Figure 6b)***, the separation between the two groups are mainly related to lactate, glucose and several amino acids. The same results were obtained for the male individual

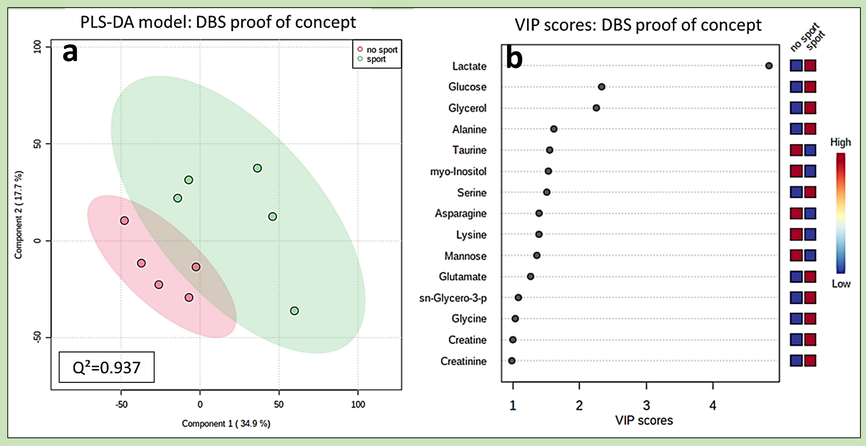

Figure 6: (a) PLS-DA model of the woman for "sport" and "no sport" conditions (Q^2=0.937); **(b)** VIP scores related to previous PLS-DA model

Conclusions:

This study demonstrates that DBS sampling can be a valid alternative for untargeted NMR-based metabolomics analysis of blood samples. Indeed , even if the bacterial contamination remains a weak point to be solved, the large range of metabolites and their adequate concentration extracted, allowed the DBS sampling's application to our real case-study.

In conclusion, this study represents a good starting point for the development of an easy and poorly invasive device to collect and store blood sample in the context of personalized medicine approach.

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