Optimization of an untargeted method for metabolomics and lipidomics profiling of cells using GC×GC-TOFMS

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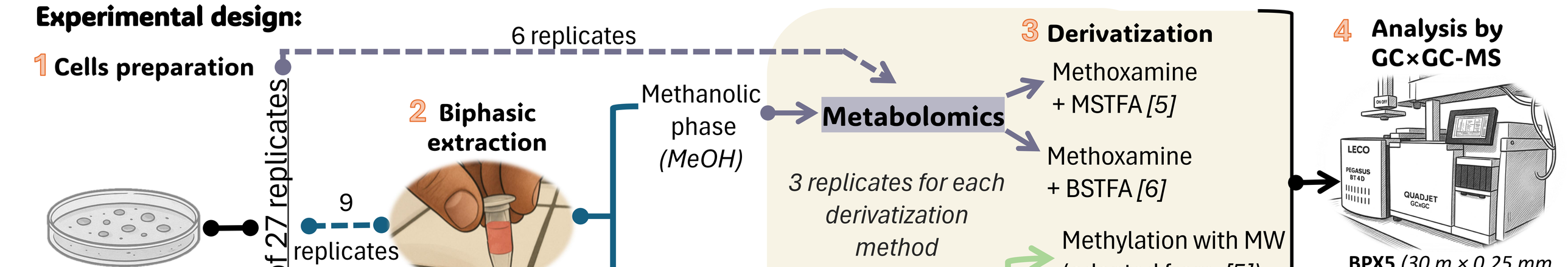
Background: Metabolites and lipids reflect the dynamic state of cells, offering critical information on biochemical pathways, stress responses, and disease mechanisms [1,2]. While metabolomics captures central metabolic activities, lipidomics can reveal alterations in membrane composition, signalling, and energy storage [3]. Despite their interconnected nature, integrated most studies focus on one domain, limiting the broader understanding of cellular function and regulation.

Aim: This study aimed to compare different derivatization methods to perform an untargeted analysis of metabolites and fatty acids (FA), directly on cells and after an extraction of the cells.

Experimental design:



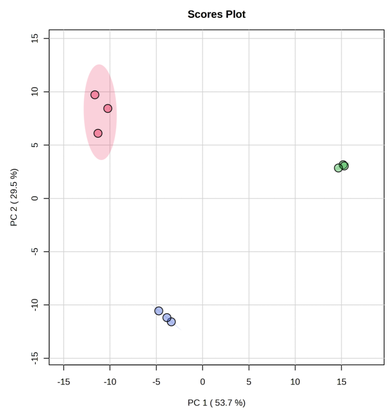
Results:

FA profile:

PCA

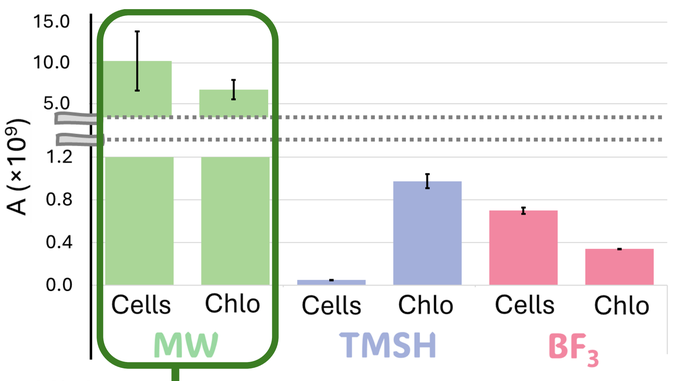
BF_3/TMSH / MW

The PCA showed a distinct separation based on all **derivatization protocols:**



<30 discriminant compounds

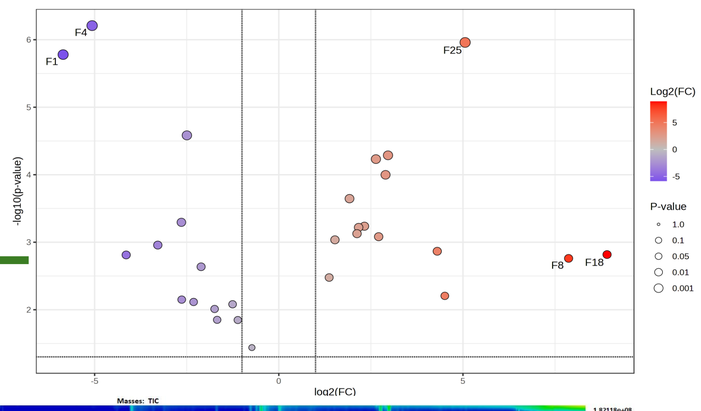
Sum of the area of all FA

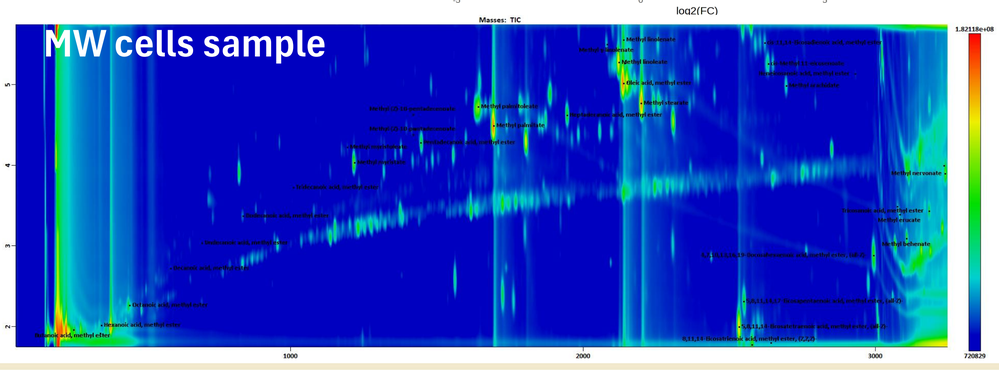


Higher intensity

Study of differences between **MW Cells/MW Chlo**

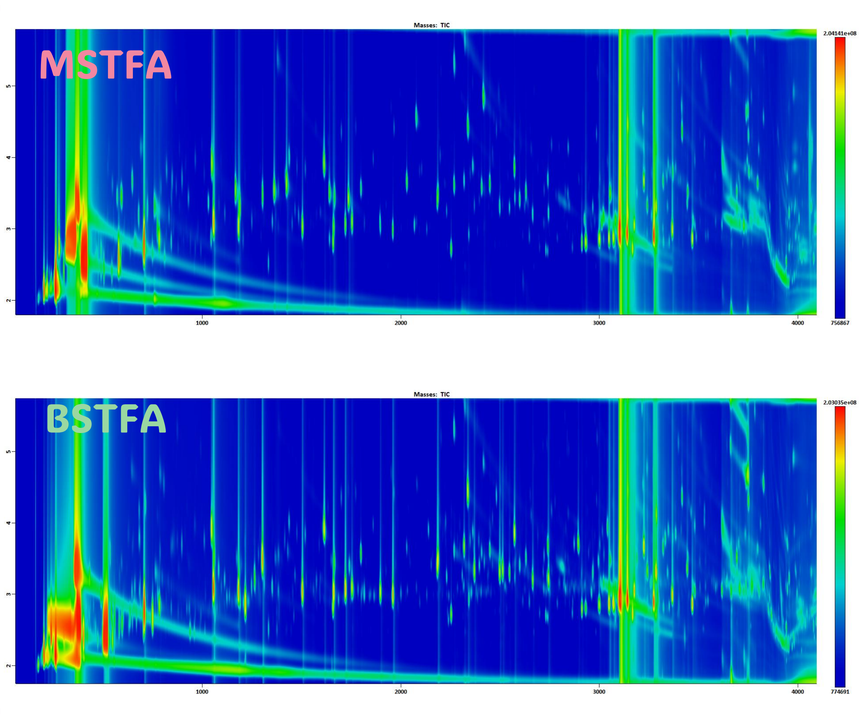
Volcano Plot





Metabolomics:

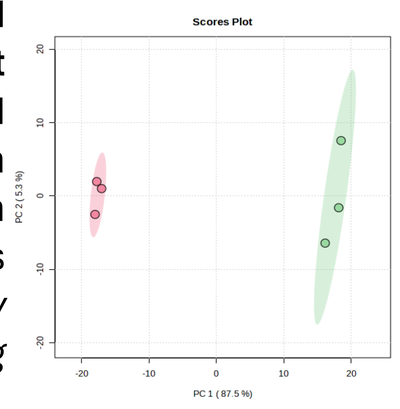
Similar profiles were observed *400 features found to be statistically discriminant*



PCA

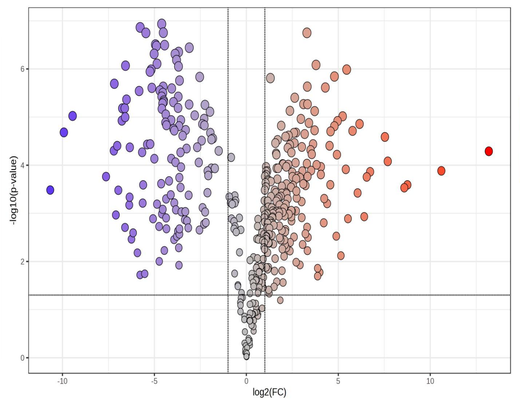
MSTFA/BSTFA

Both plots showed a distinct separation based on both derivatization protocols *~100 significantly differing*



Volcano Plot

BSTFA/MSTFA



Conclusions: GC×GC data analysis using the Tiles approach enabled targeted chromatogram segmentation, improving trend detection and reducing processing time. The results suggested that the most informative method for FA analysis was the methylation with MW. For metabolomics, derivatization with MSTFA showed better repeatability, yielding an overall higher signal, compared to BSTFA. Further investigation will be performed to compare the ability of the different workflows to extract useful discriminatory information to be applied to broader metabolism studies.

References: [1] Patti, et al. (2012) Nat Rev Mol Cell Biol. 13, 263–269; [2] Kind & Fiehn (2010) Bioanal Rev. 2, 23–60; [3] Cajka & Fiehn (2016). Anal Chem. 88, 524–545; [4] Sapcariu et al. (2014) MethodsX. 1, 74-80; [5] Ferrara et al. (2024) Chrom. B 1236, 124074; [6] Margarida et al. (2018) Arch Toxicol. 92, 3307-3323; [7] Beccaria et al. (2018) Anal Bioanal Chem 410, 7987–7996; [8] Bhatt et al. (2023) Int. J. Mol. Sci. 24, 9614

Acknowledgments: The authors thank Milestone, Trajan and LECO for their support. This work is supported by ACESSS (Academic Center of Excellence for Separation Science and Sensing).