Optimization of a lipidomic and metabolomic derivatization workflow for human cells analysis by GC×GC-TOFMS

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Two-dimensional gas chromatography (GC×GC) is a powerful analytical technique widely used for the comprehensive analysis of volatile and semi-volatile compounds, making it particularly well suited for large-scale, untargeted omics investigations. In this context, metabolomics and lipidomics are complementary disciplines that provide critical insights into cellular physiology: metabolomics reflects central metabolic activities, while lipidomics reveals changes in membrane composition, signalling pathways, and energy storage. Despite their strong interconnection, these approaches are often investigated separately, limiting a holistic understanding of cellular function.

This study aimed to evaluate sample preparation strategies enabling a comprehensive, untargeted analysis of polar metabolites and fatty acids to obtain a global metabolic–lipidomic profile from a single biological sample. Human A549 lung epithelial cells were cultured and aliquoted into samples containing one million cells. Three fatty acid methyl ester (FAME) derivatization methods were compared: microwave-assisted methylation, BF_3-catalyzed two-step esterification, and trimethylsulfonium hydroxide (TMSH) methylation. Polar metabolites were derivatized with two different reagents MSTFA and BSTFA. Cell culture media were subjected to the same derivatization protocols, and comparisons were systematically performed between culture media and media containing cells.

Among the FAME derivatization approaches, microwave-assisted methylation achieved the broadest fatty acid coverage, enabling the detection of approximately 50 fatty acids spanning C_8 to C_{26}, including monounsaturated, polyunsaturated, and branched compounds. In addition to its extended coverage, this method provided rapid and efficient methylation of complex lipids and offered practical advantages such as sealed reaction conditions, low toxicity, and reagent stability. For metabolomic profiling, MSTFA derivatization was the most effective, leading to the identification of 193 significant metabolites, with strong coverage of sugars, amino acids, and nucleosides, whereas BSTFA derivatization resulted in the detection of 85 metabolites, showing selectivity toward carboxylic acids, amines, and steroids.

These findings highlight the critical role of optimal derivatization strategies in maximizing compound coverage for GC×GC-based metabolomic and lipidomic analyses and provide a rational framework for selecting optimal derivatization workflows for cell-based applications.