# Untargeted screening of lung ex vivo samples to support metabolic pathway discovery

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## Abstract

The ballistic rise of high-resolution analytical technologies has opened a large playground for all type of untargeted “omics” screening. Comprehensive two-dimensional gas chromatography (GC×GC) has become a method of choice for complex mixture characterization. The two chromatographic dimensions and the possibility to hyphen high-speed high-resolution time-of-flight mass spectrometers (HRTOFMS) locates GC×GC as a method of choice for untargeted metabolomics. In this quest of the big picture, it is important to carefully apprehend every step of the analytical workflow from the sampling to the statistical process. The complexity of the approach cannot impact the analytical robustness.

In this study, Bronchoalveolar lavage fluid (BALF) samples were analyzed by solid phase microextraction (SPME) coupled to GC×GC-TOFMS. These samples were analyzed as part of a discovery study for lung inflammation mechanisms characterization. First, a QC mixture was designed by pooling an aliquot of the different liquid samples spiked with internal standards. This QC solution was used for optimization and daily system monitoring. Central composite design is a method of choice to establish optimal analytical conditions. For SPME, the peak intensity was used as a quality metric versus the fiber type, incubation time and temperature as variable parameters. For the GC×GC-TOFMS, normal and reversed column combinations were tested. Based on these optimal conditions, the samples were injected and the optimization was performed for the pre-processing parameters. Different alignment, normalization, and data transformation approaches were compared using unsupervised clustering.

The resulting workflow offers robust and controlled conditions for the second phase of this project.